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

STEADY STATE KINETIC ANALYSIS OF ATP HYDROLYSIS*

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The DNA-dependent ATPase activity of the recA protein of *Escherichia coli* shows a complex dependence on ATP concentration. With a single-stranded (SS) DNA cofactor, the Hill coefficient for ATP is 3.3 at pH 8.1 and 1.4 at pH 6.2. With a double-stranded (DS) DNA cofactor, the Hill coefficient is 3.3 at pH 6.2 (no reaction is detectable at pH 8.1). In the presence of SS DNA, the K_m for ATP is 20 μM, independent of pH, while with DS DNA at pH 6.2, K_m^{ATP} is 100 μM. These and other observations indicate that the interaction of recA protein with ATP is influenced by both pH and DNA cofactor.

ADP, UTP, dTTP, and GTP are competitive inhibitors of the ATPase activity of recA protein, indicating that there is a single binding site for nucleoside triphosphates. Nucleoside triphosphates, but not ADP, reduce the Hill coefficient for ATP hydrolysis and thus can contribute to the cooperative effect of ATP.

The recA protein of *Escherichia coli* promotes hybridization of SS[1] DNA either to another SS DNA molecule (annealing) (1) or to a homologous sequence within a DS DNA molecule (assimilation) (2, 3). These reactions are analogous; both involve pairing of DNA molecules and hybridization of complementary sequences. In the assimilation reaction, the interaction with DS DNA introduces additional complexity. The binding of recA protein to DS DNA requires ATP and SS DNA (4) and results in unwinding of the duplex (5–7); however, the requirement for SS DNA is obviated at pH 6.2 (6, 7). The binding of DS DNA does not require that the SS DNA be homologous. Thus, once paired, recA protein may align DNA molecules at homologous sequences. These are clearly complex processes, remarkable for a protein of molecular weight 38,000.

Both types of hybridization reactions require ATP hydrolysis. In the previous paper (8), we demonstrated that under optimal conditions for DNA binding, either SS or DS DNA can stimulate the ATPase of recA protein. As noted above, ATP is required for binding of recA protein to DS DNA. Although not required for SS DNA binding, ATP also influences the stability of recA protein-SS DNA complexes (6, 7). Furthermore, ATP has an effect on the oligomeric structure of the recA protein (6, 9). Thus, ATP serves a number of functions in the complex series of events which make up the hybridization reactions.

To gain further insight into the relationship of these effects to the mechanism of ATP hydrolysis and the DNA hybridization reactions, we have performed a steady state kinetic analysis of ATP hydrolysis. We have found that hydrolysis has an exponential dependence on ATP concentration. This dependence is complex in that it is affected both by pH and the DNA factor. These factors account for the different sensitivities to ADP of SS and DS DNA-dependent ATP hydrolysis that we had observed previously (8). In addition, we have found that UTP, which is also hydrolyzed by the recA protein, is a competitive inhibitor of the ATPase activity, consistent with the notion of a single (or overlapping) active site for nucleoside triphosphate hydrolysis. Finally, we have observed that the pH, previously found to affect DS DNA-dependent ATP hydrolysis, also affects the dependence on ATP and DNA concentration of SS DNA-dependent ATP hydrolysis.

EXPERIMENTAL PROCEDURES

All reagents and assays were as described in the previous paper (8). All velocities were determined by measuring the time course of the reaction.

RESULTS

Dependence of ATP Hydrolysis on ATP Concentration—Both SS DNA-dependent ATP hydrolysis at pH 8.1 and DS DNA-dependent ATP hydrolysis at its optimum, pH 6.2, exhibited a complex dependence on ATP concentration (Fig. 1) with Hill coefficients of about 3.3 at low ATP concentrations (Fig. 2). This finding suggests that there is cooperative binding of ATP to the recA protein. Above 50 μM ATP, both reactions had Hill coefficients of 1, indicating that the enzyme was nearly saturated with ATP. Surprisingly, when hydrolysis was examined at pH 6.2 with SS DNA, there was a much simpler ATP dependence (Fig. 1*a*), with a Hill coefficient of 1.4 at low ATP concentrations (Fig. 2). Thus, the pH, previously seen to affect binding of recA protein to DS DNA (6), also affects the ATP dependence of SS DNA-dependent hydrolysis.

The apparent K_m^{ATP} values for ATP hydrolysis catalyzed by recA protein are given in Table I. These values were determined above 50 μM ATP, where the Hill coefficient was unity, using an Eadie-Hofstee plot. Despite the pH dependence of the Hill coefficient, K_m^{ATP} for SS DNA-dependent hydrolysis was about 20 μM at 30 °C at both pH 6.2 and 8.1. K_m^{ATP} for DS DNA-dependent hydrolysis was considerably

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[1] The abbreviations used are: SS, single-stranded; DS, double-stranded; ATP(γS), adenosine-5′-*O*-(3-thiotriphosphate); NTP, nucleoside triphosphate.

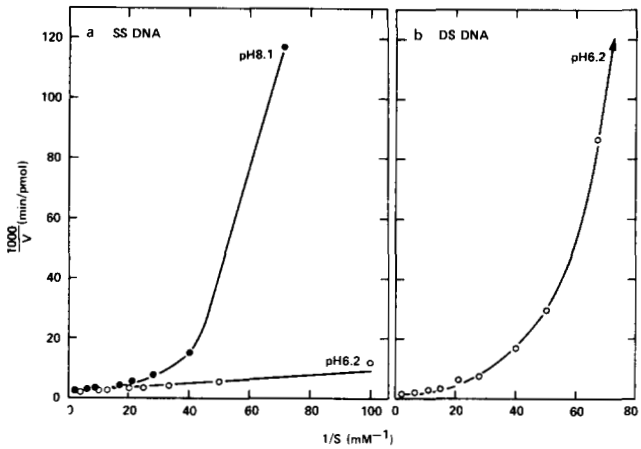

FIG. 1. **Lineweaver-Burk plot of the effect of ATP concentration on DNA-dependent hydrolysis catalyzed by the recA protein.** SS DNA-dependent ATPase reactions contained either 0.83 μM Fraction II recA protein and 22 μM φX174 SS DNA in Tris-HCl (pH 8.1) or 0.77 μM Fraction II recA protein and 84 μM φX174 SS DNA in sodium maleate (pH 6.2). DS DNA-dependent ATPase reactions contained 2.2 μM Fraction II recA protein and 26 μM pBR322 DS DNA in sodium maleate (pH 6.2).

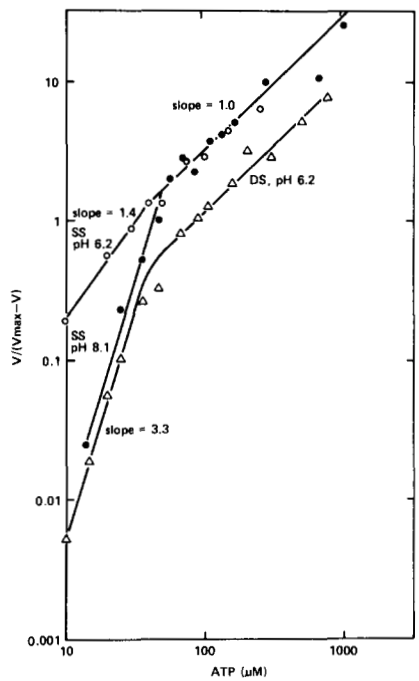

FIG. 2. **Hill plot of the effect of ATP concentration on DNA-dependent ATP hydrolysis catalyzed by the recA protein.** The data from Fig. 1 were replotted by analogy with the Hill plot for ligand binding (10, 11). V_{max} was determined from an Eadie-Hofstee plot (12).

higher, about 100 μM at 30 °C. K_m^{ATP} also showed a different dependence on temperature and enzyme concentration in the SS and DS DNA-dependent reactions. These observations suggest that the interaction of ATP with recA protein differs depending on whether SS or DS DNA is present. The turnover numbers, V_{max}/E, for SS and DS DNA-dependent hydrolysis were approximately the same.

In the absence of DNA, the reciprocal plot for the ATP dependence of hydrolysis was essentially linear at both pH 6.2 and 8.1 (Fig. 3). K_m^{ATP} was about 60 μM at pH values above 7 and 120 μM at pH 6.2, while V_{max}/E was 0.016 and 0.13 mol of ADP formed/min/mol of recA protein at high and low pH,

TABLE I

Steady state kinetic parameters for ATP hydrolysis catalyzed by the recA protein

Measurements of ATP hydrolysis were performed as described under "Experimental Procedures." At least 5 ATP concentrations were used to determine K_m^{ATP}; at each concentration, the velocity was measured from time points taken before 40% hydrolysis had occurred. K_m^{ATP} and V_{max} values were calculated from an Eadie-Hofstee plot (12). Fraction II recA protein (8) was used for DNA-dependent reactions and Fraction IV (8) for DNA-independent reactions.

DNA	pH	Temperature	recA protein	K_m^{ATP}	V_{max}/E
		°C	μM		*mol ADP/mol recA protein/min*
SS DNA					
Calf thymus	6.2	30	0.83	10	8.1
Calf thymus	6.2	31	0.88	25	9.2
φX174	6.2	32	0.77	33	10.1
Calf thymus	6.2	37	0.28	87	6.6
Calf thymus	6.2	37	0.55	40	11.8
Calf thymus	6.2	37	0.83	103	10.0
Calf thymus	6.2	37	1.38	72	10.8
Calf thymus	6.2	37	2.76	8	11.2
Calf thymus	8.1	30	0.83	24	8.9
φX174	8.1	30	0.83	18	6.9
Calf thymus	8.1	37	0.83	67	12.7
φX174	8.1	37	0.69	38	10.3
DS DNA					
P22	6.2	30	0.55	103	2.4
P22	6.2	30	1.85	110	6.1
pBR322	6.2	30	2.20	83	4.7
P22	6.2	37	0.55	1300	12.1
P22	6.2	37	0.97	265	8.3
P22	6.2	37	1.38	163	8.4
P22	6.2	37	1.80	148	10.3
P22	6.2	37	2.21	74	9.3
P22	6.2	37	2.76	118	10.4
None	6.2	32	6.65	114	0.15
	6.2	32	5.33	123	0.11
None	7.5	32	6.65	44	0.018
	8.1	32	5.33	80	0.015

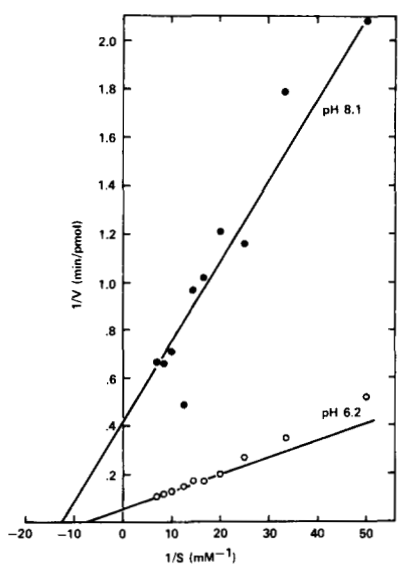

FIG. 3. **Lineweaver-Burk plot of the effect of ATP concentration on DNA-independent ATP hydrolysis catalyzed by the recA protein.** Reactions contained 5.3 μM Fraction IV recA protein in either Tris-HCl (pH 8.1) or sodium maleate (pH 6.2).

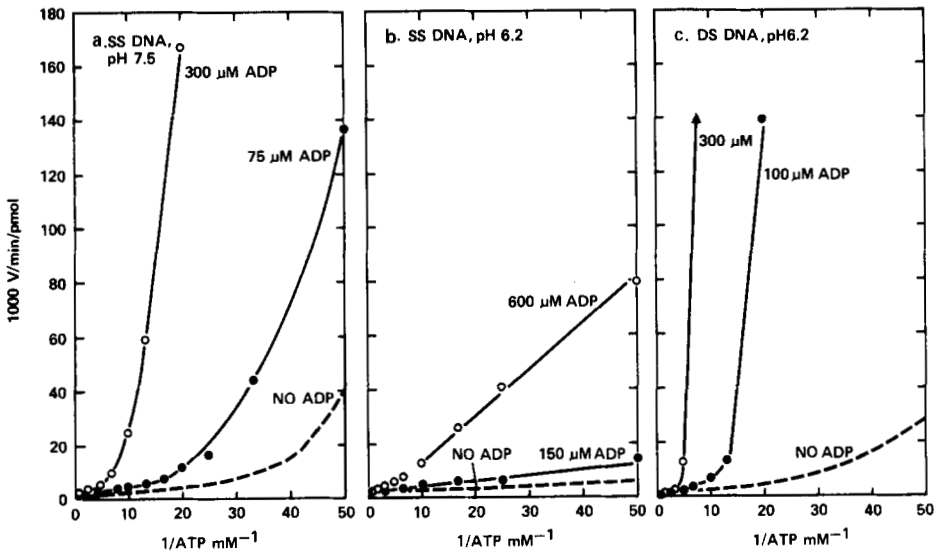

FIG. 4. **ADP inhibition of ATPase reactions catalyzed by the recA protein.** SS DNA-dependent ATPase reactions contained 84 μM φX174 SS DNA and 0.88 μM Fraction II recA protein in either Tris-HCl (pH 7.5) or sodium maleate (pH 6.2). DS DNA-dependent ATPase reactions in sodium maleate (pH 6.2) contained 77 μM pZ6b DS DNA and 2.2 μM Fraction II recA protein. The "No ADP" curves are from Fig. 1.

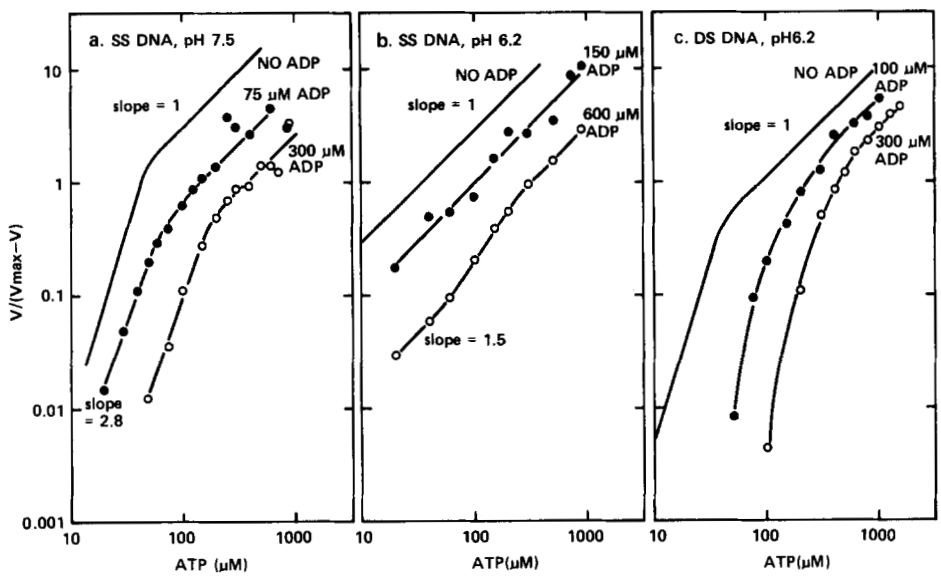

FIG. 5. **Effect of ADP on the Hill plot for ATP.** The data from Fig. 4 were replotted as in Fig. 2. V_{max} was determined from an Eadie-Hofstee plot. The "No ADP" curves are from Fig. 2.

TABLE II

Inhibition by ADP of SS and DS DNA-dependent ATPase activities of the recA protein

Reactions were performed as described in Fig. 4. V_{max} and K_i^{ADP} were determined from Eadie-Hofstee plots and the Hill coefficients from Fig. 5.

Reaction	ADP	V_{max}/E	K_m^{ATP}	K_i^{ADP}	Hill coefficient
	μM	*mol ADP/min/mol/recA protein*	μM		
SS DNA, pH 7.5	0	~9	20		3.3
	75	9.2	141	12	2.8
	300	9.2	355	18	2.8
SS DNA, pH 6.2	0	~9	20		1.4
	150	8.4	102	37	1.0
	600	8.2	349	36	1.5
DS DNA, pH 6.2	0	~6	100		3.3
	100	6.5	194	106	>2
	300	6.6	342	124	>2

respectively (Table I). Thus, both K_m^{ATP} and V_{max}/E were pH dependent in the absence of DNA.

Inhibition of Hydrolysis by ADP—ADP was an inhibitor of all 3 DNA-dependent ATP hydrolytic reactions (Fig. 4, Table II). The inhibition was competitive since V_{max} was unaffected by ADP. For SS DNA-dependent hydrolysis, K_i^{ADP} was about 15 μM at pH 7.5 and 36 μM at pH 6.2, while for DS DNA-dependent hydrolysis, K_i^{ADP} was about 115 μM. Thus, K_i^{ADP} parallels K_m^{ATP} in its dependence on reaction conditions.

The Hill coefficients for SS DNA-dependent hydrolysis at

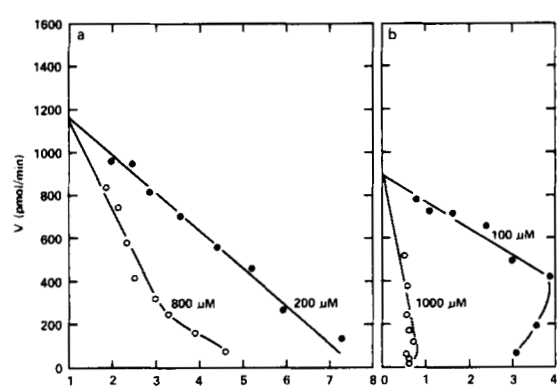

FIG. 6. **Inhibition of ATPase activity of the recA protein by UTP and dTTP.** Reactions in Tris-HCl (pH 8.1) contained 257 μM calf thymus SS DNA and 1.4 μM Fraction II recA protein and either (*a*) 200 μM or 800 μM UTP or (*b*) 100 μM or 1000 μM dTTP.

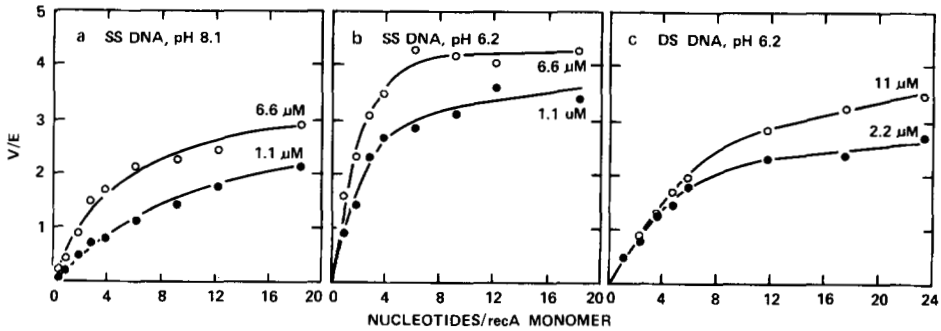

FIG. 7. **Effect of DNA concentration on the ATPase activity of the recA protein.** Reactions in either Tris-HCl (pH 8.0) or sodium maleate (pH 6.2) contained 930 μM ATP and the indicated amount of Fraction II recA protein. *a* and *b*, φX174 SS DNA. *c*, pZ6b DS DNA.

TABLE III

Inhibition of ATPase activity of recA protein by nucleoside triphosphates

SS DNA-dependent reactions were as described in Fig. 6. V_{max} and K_i were determined from an Eadie-Hofstee plot as in Fig. 6 and Hill coefficients as in Fig. 2.

NTP	Concentration	V_{max}/E	K_i^{NTP}	Hill coefficient
	μM		μM	
UTP	200	13.9	26	0.9
UTP	800	14.0	40	0.9
dTTP	100	10.7	20	
dTTP	200			~1.7
dTTP	1000	11.2	19	
GTP	723	~13	>60	~1.6

TABLE IV

Dependence of ATPase reactions catalyzed by recA protein on DNA concentration

Reactions were performed as in Fig. 7. K_m^{DNA} and V_{max} were calculated from Eadie-Hofstee plots.

DNA	pH	recA protein	K_m^{DNA}	V_{max}/E
		μM		
SS	8.1	1.1	12	6.6
		6.6	35	7.4
SS	6.2	1.1	3.2	8.6
		6.6	17	8.7
DS	6.2	1.1	3.5	3.1
		2.2	15	7.2
		11	118	11

pH 6.2 and 7.5 were not greatly affected by ADP (Fig. 5, *a* and *b*, Table II). However, ADP affected the Hill coefficient for ATP in DS DNA-dependent ATP hydrolysis (Fig. 5*c*). In the presence of ADP, the Hill coefficient was not constant but decreased gradually with increasing ATP concentration. At the lowest ATP concentrations, the Hill coefficient was greater than 4. In addition, as the ADP concentration increased, higher concentrations of ATP were required to attain a Hill coefficient of 1. These results imply that ADP does not contribute to and may, in fact, disrupt the cooperative interaction of ATP with recA protein.

Inhibition of Hydrolysis by Other Nucleoside Triphosphates—Both UTP, which is hydrolyzed by recA protein, and dTTP, which is not, behaved as inhibitors of the SS DNA-dependent ATPase reaction at pH 8 (Fig. 6). Inhibition was competitive since V_{max} was unaffected (Table III). UTP had a K_i of about 33 μM, while dTTP had a K_i of 20 μM. Unlike ADP, both UTP and dTTP caused a reduction in the Hill coefficient (Table III). Two other nonhydrolyzable nucleoside triphosphates, GTP (K_i > 60 μM) and ATP(γS) (K_i approximately 0.6 μM, Ref. 13), also reduced the Hill coefficient.

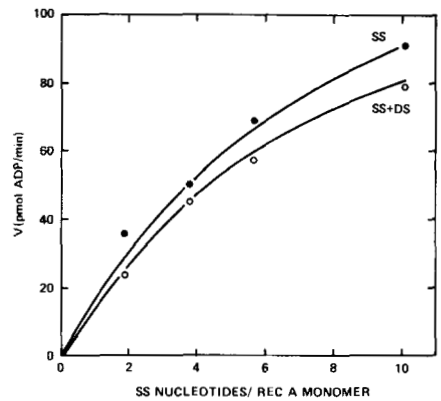

FIG. 8. **Effect of DS DNA on SS DNA-dependent ATPase activity of the recA protein.** Reactions in Tris-HCl (pH 8.0) contained 620 μM ATP, 1.1 μM Fraction IV recA protein, φX174 SS DNA as indicated, and either 51 μM or no pZ6b DS DNA.

These results suggest that other nucleoside triphosphates can contribute to the cooperative interaction of ATP with recA protein.

Dependence of ATP Hydrolysis on DNA Concentration—Titration of DNA in the various hydrolytic reactions (Fig. 7) indicated that the K_m^{DNA} values increased with increasing recA protein concentration (Table IV). This result may reflect the fact that DNA is not present in large excess over enzyme. As seen in Fig. 7, the reaction reached maximal velocity at 5 to 10 nucleotides/recA protein monomer, near the saturation value observed in DNA-binding studies (7).

Binding of DS DNA to recA protein at pH 8 requires that SS DNA be present (4) and is optimal at about 5 SS DNA nucleotides/recA monomer. Below this value, SS DNA is limiting, as observed for ATP hydrolysis. DS DNA (in excess) did not stimulate ATP hydrolysis when SS DNA was limiting and had little effect on the requirement for SS DNA (Fig. 8). This finding suggests that the initial interaction with SS DNA is rate-determining.

DISCUSSION

Two striking aspects of ATP hydrolysis catalyzed by the recA protein are the differences in K_m^{ATP} values and Hill coefficients observed with SS and DS DNA cofactors. One explanation for this behavior is that there exist different ATP binding sites on the enzyme, specific for the different ATPase reactions. However, since all reactions show the same nucleotide specificity (8) and the hydrolyzable substrates (ATP and UTP) competitively inhibit each other, it is likely that the active site for hydrolysis is the same in all ATPase reactions. The differences in the kinetic parameters must, therefore, reflect differences in the mechanism by which hydrolysis is stimulated at this active site.

The differences in the K_m^{ATP} values obtained in the presence

of SS and DS DNA are difficult to interpret for complex mechanisms. However, it is noteworthy that K_i^{ADP}, which, for competitive inhibition, reflects the ADP binding constant, parallels the K_m^{ATP}. Binding studies show that recA protein binds SS, but not DS, DNA in the absence of ATP (6). It is, therefore, plausible that ATP binds to recA protein-SS DNA complexes in the SS DNA-dependent reaction and to free recA protein in the DS DNA-dependent reaction. Thus, the different K_m^{ATP} values might reflect a difference in ATP affinity or dissociation of these forms of the recA protein.

Since the Hill coefficient is greater than 1, more than 1 ATP molecule is required/hydrolytic cycle for the maximum rate of hydrolysis. This suggests a cooperative process in which the initial binding of ATP stimulates further binding of ATP. At pH 6.2, the reduction in the Hill coefficient seen with SS DNA suggests that 1 or more of these binding steps may be bypassed. Since there is probably only a single ATP binding site per recA protein monomer (13), the observed values of the Hill coefficient imply that the active form of the enzyme is at least a trimer, consistent with earlier observations (9).

Other factors may also contribute to these characteristics. The nonlinear dependence on enzyme concentration for DS DNA-dependent hydrolysis could result in a nonlinear dependence on ATP concentration. Furthermore, binding of ATP could be important for the dissociation of ADP from the enzyme, especially in the DS DNA-dependent reaction where the extent is also affected by the ADP concentration (8).

We had previously found that, at pH 6.2, ADP is a more potent inhibitor of ATP hydrolysis in the presence of DS DNA than in the presence of SS DNA (8). Although the K_m^{ATP} values suggest weaker binding of ATP in the DS DNA-dependent reaction, it is unlikely that this can account for the differential inhibition since K_i^{ADP} is also greater in the presence of DS DNA. Rather, the sensitivity of DS DNA-dependent hydrolysis appears to be due to the nonlinear ATP dependence of this reaction as compared with SS DNA-dependent hydrolysis (compare Fig. 4, *b* and *c*). Because of the greater exponential dependence on ATP concentration with DS DNA, a reduction in bound ATP due to competition by ADP causes a disproportionate reduction in velocity when compared with SS DNA-dependent hydrolysis.

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