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 *Km* for ATP is 20 μM, independent of pH, while with DS DNA at pH 6.2, *Km* for 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, dATP, 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 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 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 the 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. 1a), 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 *Km* for 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, *Km* for SS DNA-dependent hydrolysis was about 20 μM at 30 °C at both pH 6.2 and 8.1.

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The abbreviations used are: SS, single-stranded; DS, double-stranded; ATP(s), adenosine-5′-O-(3-thiotriphosphate); NTP, nucleoside triphosphate.
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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). Vmax was determined from an Eadie-Hofstee plot (12).

higher, about 100 μM at 30 °C. KmATP 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, Vmax/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). KmATP was about 60 μM at pH values above 7 and 120 μM at pH 6.2, while Vmax/E was 0.016 and 0.13 mol of ADP formed/min/mol of recA protein at high and low pH,

TABLE I

| DNA      | pH   | Temp. | recA protein | KmATP | Vmax/E |
|----------|------|-------|--------------|-------|--------|
| SS DNA   |      |       |              |       |        |
| Calf thymus | 6.2  | 30    | 0.82         | 10    | 8.1    |
| Calf thymus | 6.2  | 37    | 0.28         | 87    | 6.6    |
| φX174    | 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    | 0.09         | 35    | 10.3   |
| DS DNA   |      |       |              |       |        |
| P22      | 6.2  | 30    | 0.55         | 103   | 2.4    |
| P22      | 6.2  | 37    | 0.55         | 1300  | 121    |
| pBR322   | 6.2  | 37    | 0.97         | 265   | 8.3    |
| P22      | 6.2  | 37    | 1.38         | 163   | 8.4    |
| P22      | 6.2  | 37    | 1.80         | 145   | 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   |
| None     | 6.2  | 32    | 5.33         | 123   | 0.11   |
| None     | 7.5  | 32    | 6.65         | 44    | 0.018  |
| None     | 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 p26b 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. Vmax 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. Vmax and K_i were determined from Eadie-Hofstee plots and the Hill coefficients from Fig. 5.

| Reaction | ADP | Vmax/E | K_i | K_i | 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      | 6.5 | 242 | 124 | >2 |

respectively (Table I). Thus, both K_i and Vmax/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 Vmax was unaffected by ADP. For SS DNA-dependent hydrolysis, K_i was about 15 μM at pH 7.5 and 36 μM at pH 6.2, while for DS DNA-dependent hydrolysis, K_i was about 115 μM. Thus, K_i parallels K_i in its dependence on reaction conditions.

The Hill coefficients for SS DNA-dependent hydrolysis at
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**Table III**
Inhibition of ATPase activity of recA protein by nucleoside triphosphates

| NTP | Concentration (μM) | Vmax (μM) | K<sub>NTP</sub> (μM) | Hill coefficient |
|-----|------------------|-----------|----------------------|-----------------|
| UTP | 200              | 13.9      | 26                   | 0.9             |
| UTP | 800              | 14.0      | 40                   | 0.9             |
| dTTP| 100              | 10.7      | 20                   | 1.7             |
| dTTP| 200              | 11.2      | 19                   | 1.7             |
| GTP | 723              | ~13       | >60                  | ~1.6            |

**Table IV**
Dependence of ATPase reactions catalyzed by recA protein on DNA concentration

| DNA | pH | recA protein | K<sub>DNA</sub> (μM) | Vmax (μM) |
|-----|----|--------------|-----------------------|-----------|
| SS  | 6.2| 1.1          | 12                    | 6.6       |
|     | 7.5| 6.6          | 35                    | 7.4       |
| SS  | 6.2| 1.1          | 3.2                   | 8.6       |
|     | 7.5| 6.6          | 17                    | 8.7       |
| DS  | 6.2| 1.1          | 3.5                   | 3.1       |
|     | 7.2| 2.2          | 15                    | 7.2       |
|     | 11 | 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. 5c). 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<sub>max</sub> was unaffected (Table III). UTP had a K<sub>i</sub> of about 33 μM, while dTTP had a K<sub>i</sub> 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<sub>i</sub> > 60 μM) and ATP(γS) (K<sub>i</sub> approximately 0.6 μM, Ref. 12), also reduced the Hill coefficient.

**Discussion**

Two striking aspects of ATP hydrolysis catalyzed by the recA protein are the differences in K<sub>m</sub><sup>ATP</sup> 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<sub>m</sub><sup>ATP</sup> values obtained in the presence...
of SS and DS DNA are difficult to interpret for complex mechanisms. However, it is noteworthy that $K_{\text{ATP}}^\text{ADP}$, which, for competitive inhibition, reflects the ADP binding constant, parallels the $K_{\text{m}}^\text{ATP}$. 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 $K_{\text{m}}^\text{ATP}$ 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 $K_{\text{m}}^\text{ATP}$ values suggest weaker binding of ATP in the DS DNA-dependent reaction, it is unlikely that this can account for the differential inhibition since $K_{\text{ATP}}^\text{ADP}$ 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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