Interaction of the recA Protein of *Escherichia coli* with Adenosine 5'-O-(3-Thiotriphosphate)*

(Received for publication, December 22, 1980, and in revised form, April 27, 1981)

George M. Weinstock†, Kevin McEntee§, and I. R. Lehman

From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Incubation of the recA protein of *Escherichia coli* with the ATP analog adenosine 5'-O-(3-thiotriphosphate) (ATP(γS)) in the presence of DNA produces an irreversible inhibition of ATPase activity, although in the presence of ATP, ATP(γS) shows an initial competitive inhibition. ATP(γS) is not appreciably hydrolyzed by recA protein and the inhibition of ATPase activity is due to the formation of stable complexes which contain equimolar amounts of ATP(γS) and recA protein. Formation of stable complexes requires DNA, which is also stably bound to recA protein in the presence of ATP(γS), at a ratio of 5 to 10 nucleotides/recA protein monomer. The DNA requirement is satisfied by either single- or double-stranded DNA, and in the latter case, the pH dependence is comparable to that observed for ATP hydrolysis. Binding of ATP(γS) is inhibited by other nucleoside diphosphates and triphosphates with efficiencies corresponding to their inhibitory effects on the ATPase activity of recA protein.

The ATP analog of adenosine 5'-O-(3-thiotriphosphate), ATP(γS), has proved an invaluable aid in studies of the recA protein of *Escherichia coli*. This derivative, in which a sulfur atom replaces one of the oxygen atoms of the γ-phosphate of ATP, is a potent inhibitor of the ATP-dependent protein and hence is useful in studies of the ATP- and GTP-dependent protease activities of the recA protein, we sought to define in detail the interaction between ATP(γS) and the recA protein. A previous study (2) showed that the inhibition of ATPase activity by ATP(γS) was competitive and that ATP(γS) was not efficiently hydrolyzed by the recA protein. Here, we extend these observations and show that although inhibition is competitive, it is also essentially irreversible due to the formation of recA protein-ATP(γS) complexes that neither hydrolyze the ATP(γS) nor dissociate during the course of most recA protein-dependent reactions. Furthermore, we find that the complexes contain 1 ATP(γS) molecule/recA protein monomer and require DNA for their formation.

**EXPERIMENTAL PROCEDURES**

Materials—[^35S]ATP(γS) was a generous gift from Dr. F. Eckstein (Max Planck Institut, Göttingen, Germany) and was also purchased from New England Nuclear. Unlabeled ATP(γS) and GTP(γS) were from Boehringer Mannheim; UTP(γS) was also generously donated by Dr. Eckstein. In various preparations, between 60 and 90% of the radioactivity of [^35S]ATP(γS) was in ATP(γS), as judged by polyethyleneimine cellulose chromatography (1) and Norit adsorption (see below). Unlabeled NTP(γS) preparations showed a similar variation in purity, depending on their age, with the major contaminant being the nucleoside diphosphate. NTP(γS) concentrations were determined using the extinction coefficient of the corresponding NTP. Other nucleoside tri- and diphosphates were obtained from P-L Biochemicals and Sigma; [^3H]ATP was from Amersham; Na₃P₂O₇·12H₂O was from Ventron (Alfa); Norit from Baker; nitrocellulose filters (HAWP, 45 mm, 24 mm diameter) from Millipore Corp.; calf thymus DNA from Sigma; poly(dT) and (dT)₁₂ from P-L Biochemicals; other DNA's were prepared as described previously (4, 10).

The recA protein was Fraction II (10), purified through the phosphocellulose step, and was greater than 90% pure. Its concentration was determined from the absorbance at 280 nm using an extinction coefficient of 1.6×10⁴ M⁻¹ cm⁻¹ at 280 nm (10). recA protein was purified through the phosphocellulose step, and was greater than 90% pure. Its concentration was determined from the absorbance at 280 nm using an extinction coefficient of 1.6×10⁴ M⁻¹ cm⁻¹ at 280 nm (10).

**Assays for Hydrolysis of ATP—** ATP hydrolysis was assayed by thin layer chromatography on polyethyleneimine cellulose plates as described previously (10). The standard reaction mixture contained 20 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 1 mM dithiothreitol, and 30 mM NaCl (B buffer). The fdter was washed once with 10 volumes of either B buffer (pH 8.0), 10 mM MgCl₂, 1 mM dithiothreitol, and 30 mM NaCl (B buffer). The fdter was washed once with 10 volumes of either B buffer.

**Filter-binding assay—** Following incubation of the standard reaction mixture (described above), an aliquot was filtered with suction through a nitrocellulose filter previously soaked in 20 mM Tris-HCl (pH 8.0), 10 mM MgCl₂, 1 mM dithiothreitol, and 30 mM NaCl (B buffer). The filter was washed once with 10 volumes of either B buffer followed by a second wash with 10 volumes of B buffer. When all liquid had passed through, the filter was dried and the bound radioactivity determined.

Filtration was routinely carried out at 2 ml/min; slower rates did not increase retention. At least 10% of recA protein could be retained by the filters and no difference in efficiency of retention was found between filtration of 20-μl or 90-μl aliquots of a 27 μM solution.
of recA protein. Filters pretreated with 0.5 m KOH (9) showed a similar capacity. However, pretreatment of the protein with either 1% SDS or Sarkosyl for 2 min at 30 °C completely eliminated its capacity to bind to filters. Protein binding to filters did not require Mg²⁺ and was stable to repeated washing with B buffer.

**RESULTS**

*Irreversible Inhibition of ATPase by ATP(γS)*—ATP(γS) inhibits annealing of complementary single strands of DNA, single strand assimilation into homologous DS DNA, and the DNA-dependent ATPase activities associated with the recA protein. Inhibition of the SS DNA-dependent ATPase by ATP(γS) appeared competitive (Ref. 2 and Fig. 1) with a $K_{app}$ of approximately 0.6 μM as contrasted with a $K_a$ for ATP of approximately 20 μM (12). ATP(γS) also inhibited the DS DNA-dependent ATPase and the UTPase activities of recA protein (data not shown).

ATP(γS) inhibited the extent as well as the initial rate of ATP hydrolysis, suggesting that in the presence of ATP(γS) the enzyme was irreversibly altered. To test this possibility, recA protein was preincubated with varying concentrations of ATP(γS) in the presence of SS DNA, then ATP was added, and the ATP(γS) concentration was adjusted so that it remained constant and ATPase activity was measured. As shown in Fig. 2, preincubation with increasing concentrations of ATP(γS) led to a corresponding increase in inhibition of the ATPase. The degree of inhibition was directly proportional to the amount of ATP(γS) present during preincubation. Thus, since the ATP(γS) concentration was constant during ATP hydrolysis, inhibition must have occurred during the preincubation. Approximately 70% of the ATPase activity could be inhibited under these conditions, although >99% of the ATPase activity was due to recA protein. Maximum inhibition occurred when there were equimolar concentrations of ATP(γS) and recA protein. Thus, ATP(γS) causes an irreversible inhibition of the ATPase activity of the recA protein.

The irreversible inhibition required DNA. As shown in Fig. 3, preincubation of recA protein with excess ATP(γS) in the presence of varying amounts of SS DNA led to a progressive inhibition of ATPase activity, dependent on the ratio of DNA nucleotides to recA protein. Maximum inhibition occurred at a ratio of about 9 nucleotides/recA protein monomer. When recA protein was preincubated with ATP(γS) in the presence of DS DNA at pH 6.2, inhibition of both DS and SS DNA-dependent ATPase activities occurred, whereas at pH 8.0, there was no inhibition, consistent with the pH optimum for DS DNA-dependent ATP hydrolysis and DS DNA binding by the recA protein (8, 10). Preincubation in the absence of DNA gave no inhibition of DNA-independent ATPase activity at either pH 6.2 or pH 7.5.

These findings indicate that the irreversible inhibition of ATPase activity by ATP(γS) has requirements similar to those for ATP hydrolysis, i.e., a DNA cofactor and the appropriate pH in the presence of DS DNA. These observations suggest 3 possible mechanisms for the inhibition: (i) ATP(γS) is hydrolyzed, but the thiophosphate produced is tightly bound to the enzyme and dissociates very slowly; (ii) hydrolysis is initiated and a covalent recA protein-ATP(γS) or recA protein-P-O₅S intermediate accumulates; or (iii) no bonds are broken, but a stable noncovalent recA protein-ATP(γS) complex is formed. The experiments described below favor the 3rd alternative.

*Lack of Hydrolysis of ATP(γS) by recA Protein—Incubation with ATP(γS) inhibited the extent as well as the initial rate of ATP hydrolysis, suggesting that in the presence of ATP(γS) the enzyme was irreversibly altered. To test this possibility, recA protein was preincubated with varying concentrations of ATP(γS) in the presence of SS DNA, then ATP was added, and the ATP(γS) concentration was adjusted so that it remained constant and ATPase activity was measured. As shown in Fig. 2, preincubation with increasing concentrations of ATP(γS) led to a corresponding increase in inhibition of the ATPase. The degree of inhibition was directly proportional to the amount of ATP(γS) present during preincubation. Thus, since the ATP(γS) concentration was constant during ATP hydrolysis, inhibition must have occurred during the preincubation. Approximately 70% of the ATPase activity could be inhibited under these conditions, although >99% of the ATPase activity was due to recA protein. Maximum inhibition occurred when there were equimolar concentrations of ATP(γS) and recA protein. Thus, ATP(γS) causes an irreversible inhibition of the ATPase activity of the recA protein.

The irreversible inhibition required DNA. As shown in Fig. 3, preincubation of recA protein with excess ATP(γS) in the presence of varying amounts of SS DNA led to a progressive inhibition of ATPase activity, dependent on the ratio of DNA nucleotides to recA protein. Maximum inhibition occurred at a ratio of about 9 nucleotides/recA protein monomer. When recA protein was preincubated with ATP(γS) in the presence of DS DNA at pH 6.2, inhibition of both DS and SS DNA-dependent ATPase activities occurred, whereas at pH 8.0, there was no inhibition, consistent with the pH optimum for DS DNA-dependent ATP hydrolysis and DS DNA binding by the recA protein (8, 10). Preincubation in the absence of DNA gave no inhibition of DNA-independent ATPase activity at either pH 6.2 or pH 7.5.

These findings indicate that the irreversible inhibition of ATPase activity by ATP(γS) has requirements similar to those for ATP hydrolysis, i.e., a DNA cofactor and the appropriate pH in the presence of DS DNA. These observations suggest 3 possible mechanisms for the inhibition: (i) ATP(γS) is hydrolyzed, but the thiophosphate produced is tightly bound to the enzyme and dissociates very slowly; (ii) hydrolysis is initiated and a covalent recA protein-ATP(γS) or recA protein-P-O₅S intermediate accumulates; or (iii) no bonds are broken, but a stable noncovalent recA protein-ATP(γS) complex is formed. The experiments described below favor the 3rd alternative.

*Lack of Hydrolysis of ATP(γS) by recA Protein—Incubation with ATP(γS) inhibited the extent as well as the initial rate of ATP hydrolysis, suggesting that in the presence of ATP(γS) the enzyme was irreversibly altered. To test this possibility, recA protein was preincubated with varying concentrations of ATP(γS) in the presence of SS DNA, then ATP was added, and the ATP(γS) concentration was adjusted so that it remained constant and ATPase activity was measured. As shown in Fig. 2, preincubation with increasing concentrations of ATP(γS) led to a corresponding increase in inhibition of the ATPase. The degree of inhibition was directly proportional to the amount of ATP(γS) present during preincubation. Thus, since the ATP(γS) concentration was constant during ATP hydrolysis, inhibition must have occurred during the preincubation. Approximately 70% of the ATPase activity could be inhibited under these conditions, although >99% of the ATPase activity was due to recA protein. Maximum inhibition occurred when there were equimolar concentrations of ATP(γS) and recA protein. Thus, ATP(γS) causes an irreversible inhibition of the ATPase activity of the recA protein.

The irreversible inhibition required DNA. As shown in Fig. 3, preincubation of recA protein with excess ATP(γS) in the presence of varying amounts of SS DNA led to a progressive inhibition of ATPase activity, dependent on the ratio of DNA nucleotides to recA protein. Maximum inhibition occurred at a ratio of about 9 nucleotides/recA protein monomer. When recA protein was preincubated with ATP(γS) in the presence of DS DNA at pH 6.2, inhibition of both DS and SS DNA-dependent ATPase activities occurred, whereas at pH 8.0, there was no inhibition, consistent with the pH optimum for DS DNA-dependent ATP hydrolysis and DS DNA binding by the recA protein (8, 10). Preincubation in the absence of DNA gave no inhibition of DNA-independent ATPase activity at either pH 6.2 or pH 7.5.

These findings indicate that the irreversible inhibition of ATPase activity by ATP(γS) has requirements similar to those for ATP hydrolysis, i.e., a DNA cofactor and the appropriate pH in the presence of DS DNA. These observations suggest 3 possible mechanisms for the inhibition: (i) ATP(γS) is hydrolyzed, but the thiophosphate produced is tightly bound to the enzyme and dissociates very slowly; (ii) hydrolysis is initiated and a covalent recA protein-ATP(γS) or recA protein-P-O₅S intermediate accumulates; or (iii) no bonds are broken, but a stable noncovalent recA protein-ATP(γS) complex is formed. The experiments described below favor the 3rd alternative.
Interaction of recA Protein with ATP(γS)

The standard reaction (250 μl) with 20 mM Tris-HCl (pH 8.0) contained 2.1 μM [35S]ATP(γS), 121 μM φX174 SS DNA, and 5.3 μM recA protein. After incubation for 30 min at 30 °C, 30-μl aliquots were assayed by filter binding (low salt wash) or Norit adsorption as described under "Experimental Procedures" before and after a 2-min incubation at 60 °C.

| Per cent of total radioactivity | Complete | SS DNA | recA protein |
|--------------------------------|----------|--------|--------------|
| Initially bound to filter      | 53       | 2.0    | 0.4          |
| Bound to filter after 2 min, 60 °C | 0.9      | 1.5    | 0.8          |
| Initially Norit nonadsorbable  | 9.5      | 9.4    | 5.6          |
| Norit nonadsorbable after 2 min, 60 °C | 11.5    | 12.1   | 6.3          |

 conversion required recA protein and either SS or DS DNA, although DS DNA was effective only at a pH at which the DS DNA-dependent ATPase was active. Retention on nitrocellulose filters was due to binding of recA protein to the filter since alkali-treated filters that have lost the capacity to bind DNA (9) were equally effective in this assay. Thus, the γS in [35S]ATP(γS) exists in a complex with recA protein that can be retained on nitrocellulose filters in the presence of 1 M NaCl. Furthermore, the conditions for formation of this complex parallel those required for irreversible inhibition of ATPase activity.

Incubation of the complexes at 60 °C for 2 min prior to filtration resulted in loss of the bound 35S (Table II), although recA protein was still efficiently retained by the filters under these conditions (data not shown). The treatment at 60 °C did not produce inorganic PO₃S as judged by Norit adsorption.
Interaction of recA Protein with ATP(γS)

8853

Fig. 5. Kinetics of formation of stable ATP(γS)-recA protein complexes at 37 °C. The standard reaction (200 μl) with 20 mM Tris-HCl (pH 8.0) or sodium maleate (pH 6.2) also contained 5 μM [35S]ATP(γS), 308 μM calf thymus SS DNA, and 1.1 μM recA protein. Twenty-five μl aliquots were assayed by filter binding (high salt wash) after the indicated times of incubation at 37 °C. O—O, Reaction was preincubated for 12 min at 37 °C without ATP(γS); ——, no preincubation.

Fig. 6. Kinetics of ATP(γS) binding to recA protein at 30 °C. The standard reaction (200 μl) with either 30 mM Tris-HCl (pH 8.0) or sodium maleate (pH 6.2) contained 4.3 μM [35S]ATP(γS), 26 μM recA protein, and either 50 μM ϕX174 SS DNA, 51 μM pβ6h DS DNA, or no DNA. Aliquots (30 μl) were assayed by filter binding (low salt wash). ●—●, SS DNA, pH 8.0; ○—○, DS DNA, pH 6.2; ▲—▲, DS DNA, pH 8.0; ■—■, no DNA, pH 8.0; □—□, no DNA, pH 6.2.

Thus, we conclude that ATP(γS) is not hydrolyzed by recA protein; furthermore, there is no accumulation of a covalent recA protein-PO₄ intermediate. Rather, the complexes consist of ATP(γS) tightly bound to recA protein. The rather mild temperature required for complete dissociation of the [35S]ATP(γS) suggests a noncovalent association.

Characteristics of the Reaction Leading to Tight Binding of ATP(γS) to recA Protein—Although sensitive to heat treatment, the recA protein-ATP(γS) complexes were otherwise extremely stable, being resistant to exhaustive washing with 1 M NaCl and exposure to 30 mM EDTA. As shown in Fig. 4, the complexes had a half-life of about 90 min at 37 °C. Their remarkable stability accounts for the irreversibility of the inhibition of ATP hydrolysis by ATP(γS).

Although the turnover number for SS DNA-dependent ATP hydrolysis at 37 °C is about 10 ADP produced/min/recA protein monomer, 1 to 2 min were required for complete binding of ATP(γS) to recA protein (Fig. 5). At 30 °C, binding of ATP(γS) in the presence of SS DNA was slightly slower, while in the presence of DS DNA (pH 6.2), the rate was much reduced (Fig. 6). In the presence of 20 μM recA protein and 30 μM ATP(γS), the DS DNA-dependent reaction showed kinetics similar to the reaction in the presence of SS DNA (data not shown). These results indicate that the high affinity of recA protein for ATP(γS) is not a consequence of a rapid rate of formation of recA protein-ATP(γS) complexes, but is rather a result of their very slow rate of dissociation and, in fact, the rate of formation of these complexes is slower than the rate of turnover of ATP during hydrolysis.

Incubation in the absence of DNA at pH 6.2 or 8.0 produced...
Interaction of recA Protein with ATP(γS)

No increase in ATP(γS) binding over a period of at least 120 min, consistent with the failure of ATP(γS) to inhibit irreversibly the DNA-independent ATPase activity of recA protein. Furthermore, no stable binding of ATP(γS) was observed in the presence of DS DNA at pH 8.0.

As shown in Table III, the formation of stable recA protein-ATP(γS) complexes required Mg²⁺. As noted earlier, once formed they were resistant to EDTA. Formation of the stable complex was also sensitive to salt, although once formed, the final complexes were stable to 1 M NaCl. Unlike the ATPase reaction (4), formation of complexes was not inhibited by N-ethylmaleimide. The polynucleotide requirement for complex formation showed the same specificity as for ATP hydrolysis (19); in particular, (dT)₂₂ did not promote complex formation but (dT)₁₀₀ did (Table III), indicating a similar polynucleotide size requirement as for ATP hydrolysis. As shown in Fig. 7, tight binding of ATP(γS) depended on the ratio of recA protein to DNA, and saturation occurred at about 6 nucleotides/recA protein monomer with either SS or DS DNA, a value similar to that observed for the irreversible inhibition of ATPase activity. At pH 8.0, DS DNA (29 nucleotides/recA protein monomer) in the presence of subsaturating amounts of SS DNA (either 2 or 5 nucleotides/recA protein monomer) caused no additional binding of ATP(γS) over the SS DNA level, although under these conditions, binding of recA protein to DS DNA is stimulated by SS DNA (2, 9).

Stoichiometry of ATP(γS) Binding to recA Protein—Titration of recA protein in the presence of excess ATP(γS) (Fig. 8) indicated that about 1.3 mol of ATP(γS) were bound/mol of recA protein monomer at 37 °C. Eighty per cent of the labeled ATP(γS) was bound when recA protein was in excess over ATP(γS). When the titration was performed at 30 °C, 1.0 mol of ATP(γS) was bound/mol of recA protein monomer (data not shown). Titration of ATP(γS) in the presence of a constant amount of recA protein (Fig. 9) gave a ratio of 1.6 mol of ATP(γS) bound/mol of recA protein monomer, although in this case, only 60% of the labeled ATP(γS) was bound. In similar titrations, at 30 °C, ratios of 1.3 and 0.7 were observed with 2.6 and 22 μM recA protein, respectively. Finally, when recA protein was preincubated with varying amounts of unlabeled ATP(γS) in the presence of DNA and

no increase in ATP(γS) binding over a period of at least 120 min, consistent with the failure of ATP(γS) to inhibit irreversibly the DNA-independent ATPase activity of recA protein. Furthermore, no stable binding of ATP(γS) was observed in the presence of DS DNA at pH 8.0.

As shown in Table III, the formation of stable recA protein-ATP(γS) complexes required Mg²⁺. As noted earlier, once formed they were resistant to EDTA. Formation of the stable complex was also sensitive to salt, although once formed, the final complexes were stable to 1 M NaCl. Unlike the ATPase reaction (4), formation of complexes was not inhibited by N-ethylmaleimide. The polynucleotide requirement for complex formation showed the same specificity as for ATP hydrolysis (19); in particular, (dT)₂₂ did not promote complex formation but (dT)₁₀₀ did (Table III), indicating a similar polynucleotide size requirement as for ATP hydrolysis. As shown in Fig. 7, tight binding of ATP(γS) depended on the ratio of recA protein to DNA, and saturation occurred at about 6 nucleotides/recA protein monomer with either SS or DS DNA, a value similar to that observed for the irreversible inhibition of ATPase activity. At pH 8.0, DS DNA (29 nucleotides/recA protein monomer) in the presence of subsaturating amounts of SS DNA (either 2 or 5 nucleotides/recA protein monomer) caused no additional binding of ATP(γS) over the SS DNA level, although under these conditions, binding of recA protein to DS DNA is stimulated by SS DNA (2, 9).

Stoichiometry of ATP(γS) Binding to recA Protein—Titration of recA protein in the presence of excess ATP(γS) (Fig. 8) indicated that about 1.3 mol of ATP(γS) were bound/mol of recA protein monomer at 37 °C. Eighty per cent of the labeled ATP(γS) was bound when recA protein was in excess over ATP(γS). When the titration was performed at 30 °C, 1.0 mol of ATP(γS) was bound/mol of recA protein monomer (data not shown). Titration of ATP(γS) in the presence of a constant amount of recA protein (Fig. 9) gave a ratio of 1.6 mol of ATP(γS) bound/mol of recA protein monomer, although in this case, only 60% of the labeled ATP(γS) was bound. In similar titrations, at 30 °C, ratios of 1.3 and 0.7 were observed with 2.6 and 22 μM recA protein, respectively. Finally, when recA protein was preincubated with varying amounts of unlabeled ATP(γS) in the presence of DNA and
the filter-binding measurements quantitatively detect all stably bound ATP(γS).

Effect of Other NTP and NDPs on Binding of ATP(γS) to recA Protein—ADP was more effective than ATP as an inhibitor of the binding of ATP(γS) to recA protein (Table IV). Since hydrolysis of ATP must have occurred during the incubation, the inhibition observed in the presence of ATP may have been due to ADP rather than ATP. Although UTP showed a weak inhibition of ATP(γS) binding, UTP(γS) and UDP were effective inhibitors. UTP(γS) is also an inhibitor of the ATPase activity of recA protein, a finding that is consistent with other observations that ATP and UTP hydrolysis involve common or overlapping binding sites (12, 13). dTTP, although not hydrolyzed to a significant extent by recA protein, was an inhibitor of ATP(γS) binding in addition to being an inhibitor of ATPase activity (12). GTP and GTP(γS), which are poorly hydrolyzed by the recA protein and are weak inhibitors of ATPase activity, had a correspondingly weak effect on binding of ATP(γS) to recA protein.

DISCUSSION

Tight Binding of ATP(γS) to recA Protein—Our main conclusion is that in the presence of DNA, ATP(γS) binds essentially irreversibly to the recA protein. Although the anhydride linkage between the β and γ phosphates in ATP(γS) could, in principle, be hydrolyzed in a manner analogous to ATP, we find little hydrolysis of ATP(γS) by recA protein. Thus, stable recA protein-ATP(γS) complexes are formed which inhibit the ATPase, UTPase, and single strand annealing and assimilation activities of recA protein.

The stable binding of ATP(γS) to recA protein is not inconsistent with its competitive inhibition of ATPase activity (2). At the ATP(γS) concentrations used, stable complex formation is a relatively slow process when compared with ATP hydrolysis. Thus, when ATP is present in excess over ATP(γS), initially at least, competitive inhibition is to be expected. However, binding of ATP(γS) will ultimately block all ATP binding sites, limiting the extent of hydrolysis. Consistent with this, we find that ATP (and ADP) inhibit the binding of ATP(γS) to recA protein. Similar results have been observed for UTP hydrolysis which shares part or all of the ATP binding site (10).

It is noteworthy that the stable binding of ATP(γS) to recA protein requires DNA. Binding of ATP(γS) to recA protein does occur in the absence of DNA, resulting in an altered oligomeric form of the protein (8), but these recA protein-ATP(γS) complexes are less stable than those formed in the presence of DNA. Since ATP(γS) also stabilizes recA protein-DNA complexes (8), the stable complexes most likely contain DNA as well as ATP(γS). Presumably, when ATP replaces ATP(γS), ATP hydrolysis alters the recA protein-ATP-DNA complexes to allow DNA annealing and assimilation to occur.

Stoichiometry of ATP(γS) Binding to recA Protein—Approximately 1 ATP(γS) molecule is bound stably/monomer of recA protein at saturation. The values from different experiments range from 0.5 to 1.7. This ratio is subject to several sources of uncertainty. These include lack of knowledge of the true extinction coefficient for recA protein, the fraction of active enzyme molecules, and the presence of impurities in the ATP(γS) preparations, for example, ADP. Despite these uncertainties, our best estimate is that there is a 1:1 molar ratio of recA protein to ATP(γS) in the stable complexes.

Since ATP(γS) irreversibly inhibits ATPase activity, and ATP(γS) binding is inhibited by both NTPs and NDPs in a manner analogous to their effects on ATPase activity, it is likely that the tight binding site for ATP(γS) is also the binding site for ATP in the ATPase reaction. In view of the Hill coefficient of 3 for ATP hydrolysis (12), the actual form of the protein in ATP hydrolysis is thus at least a trimer.

However, this does not rule out the existence of other NTP binding sites on the enzyme.

REFERENCES

1. Goody, R. S., and Eckstein, F. (1971) J. Am. Chem. Soc. 93, 6282-6287
2. Shibata, T., Cunningham, R. P., DasGupta, C., and Radding, C. M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5100-5104
3. McIntee, K., Weinstock, G. M., and Lehman, I. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2615-2619
4. Weinstock, G. M., McIntee, K., and Lehman, I. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 126-130
5. Roberts, J. W., Roberts, C. W., Craig, N. L., and Phizicky, E. M. (1978) Cold Spring Harbor Symp. Quant. Biol. 43, 917-920
6. Craig, N. L., and Roberts, J. W. (1980) Nature 283, 26-30
7. Cunningham, R. P., Shibata, T., DasGupta, C., and Radding, C. M. (1979) Nature 281, 191-195
8. McIntee, K., Weinstock, G. M., and Lehman, I. R. (1981) J. Biol. Chem. 256, 8835-8844
9. McIntee, K., Weinstock, G. M., and Lehman, I. R. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 857-861
10. Weinstock, G. M., McIntee, K., and Lehman, I. R. (1981) J. Biol. Chem. 256, 8829-8834
11. Horii, T., Ogawa, T., and Ogawa, H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 313-317
12. Weinstock, G. M., McIntee, K., and Lehman, I. R. (1981) J. Biol. Chem. 256, 8845-8849
13. Weinstock, G. M., McIntee, K., and Lehman, I. R. (1981) J. Biol. Chem. 256, 8856-8858