ATP-Stimulated Hydrolysis of GTP by RecA Protein: Kinetic Consequences of Cooperative RecA Protein–ATP Interactions†

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ABSTRACT: The cooperativity of the single-stranded DNA dependent nucleoside triphosphatase activity of the recA protein was investigated by examining the influence of a good substrate (ATP) on the hydrolysis of a poor substrate (GTP). At pH 7.5 and 37 °C, both ATP and GTP are hydrolyzed with a turnover number of 17.5 min^{-1}. The $S_{0.5}$ for GTP (750 μM), however, is nearly 20-fold higher than the $S_{0.5}$ for ATP (45 μM). Low concentrations of ATP activate the GTPase activity of the recA protein by lowering the $S_{0.5}$ for GTP; in the presence of 50 μM ATP, the $S_{0.5}$ for GTP is reduced from 750 μM to 200 μM. Concentrations of ATP greater than 50 μM result in competitive inhibition of the ATP-activated GTPase activity. Although GTP is a substrate for hydrolysis, it will not substitute for ATP as a high-energy cofactor in the standard recA protein promoted three-strand exchange reaction. To account for these results, a minimal kinetic model is presented in which ATP binding induces specific conformational changes in the recA protein that do not occur with GTP binding.

The recA protein of *Escherichia coli* (M_r 38 000) is essential for homologous genetic recombination and the postreplicative repair of damaged DNA. The purified recA protein binds cooperatively to ssDNA,[1] forming a polymeric filament-like structure. This recA–ssDNA complex catalyzes the hydrolysis of ATP to ADP and inorganic phosphate. In addition, the recA protein will promote a variety of ATP-dependent DNA-pairing activities that presumably reflect in vivo functions (Radding, 1982). These reactions include the assimilation of linear single strands into duplex DNA (D-loop formation) and the exchange of strands between linear duplex and homologous circular single-stranded DNAs (three-strand exchange). Although ATP has been shown to affect the ssDNA binding properties of the recA protein (McEntee et al., 1981; Bryant et al., 1985; Menetski & Kowalczykowski, 1985), the exact mechanistic role of ATP binding and hydrolysis in the recA protein promoted DNA-pairing reactions is not known. The ssDNA-dependent ATPase activity of the recA protein exhibits a sigmoidal dependence on ATP concentration, suggesting that ATP interacts cooperatively with the recA–ssDNA complex (Weinstock et al., 1981a). Although either ATP or dATP is believed to be the natural cofactor for recA protein dependent recombination functions, the ssDNA-dependent hydrolysis of other nucleoside triphosphates by the recA protein has been noted. The relative order of activity has been reported as ATP > UTP > CTP > GTP, with only GTP being resistant to hydrolysis (Weinstock et al., 1981b). In this paper, we examine the effect of ATP on the kinetics of GTP hydrolysis as a means of analyzing the cooperative behavior of the ssDNA-dependent nucleoside triphosphatase activity of the recA protein.

Experimental Procedures

Materials

E. coli recA protein was purified to homogeneity as previously described (Cox et al., 1981). The concentration of recA protein in stock solutions was determined by absorbance at 280 nm, using an extinction coefficient of $E^{1\%}_{280}$ = 5.9 (Craig & Roberts, 1981). ATP, GTP, and AMP-PNP were from Sigma. [^{3}H]ATP and [^{3}H]GTP were from ICN. *E. coli* SSB was provided by Dr. Roger McMacken (this department) or was from Pharmacia.

Circular ϕx ssDNA [(+) strand] and linear ϕx dsDNA were prepared as previously described (Cox & Lehman, 1981). DNA concentrations were calculated by using A_{260} of 1 as equivalent to 36 μg/mL ssDNA or 50 μg/mL dsDNA. All DNA concentrations are expressed as total nucleotides.

Methods

NTPase Assay. ATP and GTP hydrolysis were measured by using a thin-layer chromatography method as previously described (Weinstock et al., 1979). Standard NTPase assay mixtures contained 25 mM Tris-HCl (pH 7.5) [or 25 mM Bis-Tris-HCl (pH 6.2) where indicated], 10 mM $MgCl_2$, 5% glycerol, 1 mM DTT, 30 μM ϕx ssDNA, 1.0 μM recA protein, and the indicated concentrations of ATP and GTP. All reactions were initiated by the addition of nucleoside triphosphate after preincubation of all other components for 10 min at 37 °C. All reactions were carried out at pH 7.5 and 37 °C unless otherwise indicated.

Three-Strand Exchange Reactions. Three-strand exchange reactions were carried out as described by Cox and Lehman (1981). Reaction mixtures contained 25 mM Tris-HCl (pH 7.5) or 25 mM Bis-Tris-HCl (pH 6.2), 10 mM $MgCl_2$, 5% glycerol, 1 mM DTT, 3.3 μM circular ϕx ssDNA, 5.6 μM linear ϕx dsDNA, 2.0 μM recA protein, 0.3 μM SSB protein, and the indicated concentrations of ATP and GTP. Reactions were initiated by the simultaneous addition of SSB and nu-

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[1] Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ϕx, bacteriophage ϕx174; AMP-PNP, adenyl-5′-yl imidodiphosphate; SSB, *E. coli* single-stranded DNA binding protein; SDS, sodium dodecyl sulfate; NTP, nucleoside triphosphate; DTT, dithiothreitol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Bis-Tris-HCl, [bis(2-hydroxyethyl)amino]tris(hydroxymethyl)methane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

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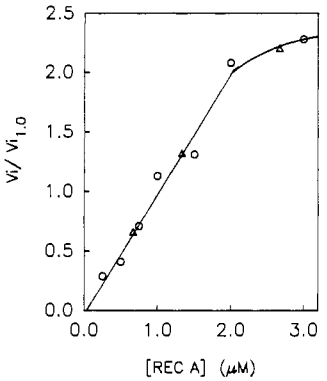


FIGURE 1: Dependence of ATP hydrolysis and GTP hydrolysis on recA protein concentration. Reactions were carried out as described under Experimental Procedures. Reaction solutions contained 30 μM ϕx ssDNA, the indicated amount of recA protein, and either 200 μM [^{3}H]ATP or 1000 μM [^{3}H]GTP. The points represent initial rates (V_i) of ATP hydrolysis (circles) or GTP hydrolysis (triangles) expressed relative to the hydrolysis rates measured at 1.0 μM recA protein ($V_{i,1.0}$).

cleoside triphosphate after preincubation of all other components for 10 min at 37 °C. All reactions were carried out at 37 °C. The progress of the strand exchange reactions was monitored by agarose gel electrophoresis as previously described (Cox & Lehman, 1981). Aliquots (40 μL) of the above reaction mixtures were quenched at various times by the addition of 5 μL of 10% SDS. The samples were loaded onto a 0.8% agarose gel and electrophoresed in TAE buffer (40 mM Tris–acetate and 1 mM EDTA). The substrates and products were visualized by ethidium bromide staining.

The hydrolysis of ATP and GTP during the strand exchange reactions was measured by using the thin-layer chromatography assay cited above. On the basis of a ssDNA concentration of 3.3 μM and a binding stoichiometry of 1 recA monomer/4 nucleotides of ssDNA (Bryant et al., 1985), the maximum amount of recA protein that will be active as a ssDNA-dependent ATPase under strand exchange conditions is 0.82 μM. This recA protein concentration was used to calculate rates of ATP and GTP hydrolysis under strand exchange conditions.

RESULTS

Kinetics of ssDNA-Dependent Hydrolysis of ATP and GTP by RecA Protein. The ssDNA-dependent recA protein catalyzed hydrolysis of ATP and GTP was measured at pH 7.5 and 37 °C. At a fixed concentration of ϕx ssDNA (30 μM), there is a linear relationship between recA protein concentration (0.25–2 μM) and the initial rates of both ATP hydrolysis and GTP hydrolysis (Figure 1). The deviation from linearity at recA protein concentrations greater than 2.0 μM is presumably due to the inefficient binding of recA protein to regions of secondary structure in the ssDNA.[2] The standard NTPase assay conditions for the kinetic measurements described below consisted of 30 μM ϕx ssDNA and 1.0 μM recA protein to ensure that there was sufficient ssDNA to bind all of the recA protein.

The dependence of the initial rate of recA protein catalyzed ATP hydrolysis on ATP concentration was measured. The data are presented in Figure 2 as a double-reciprocal plot. At pH 7.5, the turnover number ($V_{max}/[E]_T$) for ATP hydrolysis was 17.5 min^{-1} and the $S_{0.5}$ for ATP was 45 μM.[3] The

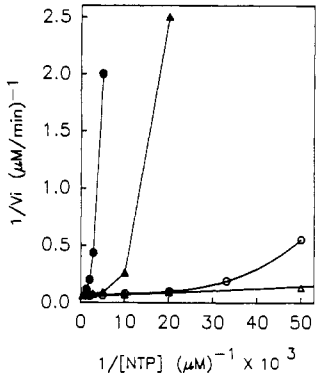


FIGURE 2: Dependence of ATP hydrolysis and GTP hydrolysis on nucleotide concentration. Reactions were carried out as described under Experimental Procedures. Reactions solutions contained either 25 mM Tris-HCl (pH 7.5) or 25 mM Bis-Tris-HCl (pH 6.2), 10 mM $MgCl_2$, 30 μM ϕx ssDNA, 1.0 μM recA protein, and the indicated concentrations of either [^{3}H]ATP or [^{3}H]GTP. The points represent the initial rates of hydrolysis of ATP, pH 7.5 (open circles); ATP, pH 6.2 (open triangles); GTP, pH 7.5 (closed circles); or GTP, pH 6.2 (closed triangles). The pH 6.2 ATP hydrolysis curve includes an additional point at 10 μM ATP.

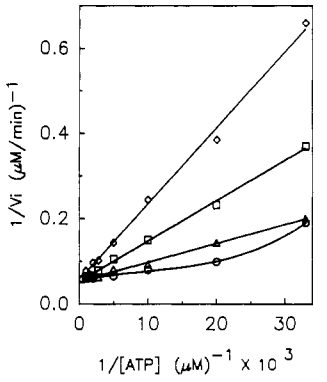


FIGURE 3: Effect of GTP on recA protein catalyzed hydrolysis of ATP. Reactions were carried out as described under Experimental Procedures. Reaction solutions contained 30 μM ϕx ssDNA, 1.0 μM recA protein, the indicated concentration of [^{3}H]ATP, and either 0 μM GTP (circles), 100 μM GTP (triangles), 500 μM GTP (squares), or 1000 μM GTP (diamonds). The points represent initial rates of ATP hydrolysis.

double-reciprocal plot is concave upward, indicating positive cooperativity for the ATPase activity with respect to ATP concentration. A Hill coefficient of 2.7 was determined at ATP concentrations near $S_{0.5}$ (0–100 μM, ATP, plot not shown). These results are consistent with a previous report (Weinstock et al., 1981a).

The dependence of the recA protein catalyzed hydrolysis of GTP on GTP concentration is also shown in Figure 2. At pH 7.5, the turnover number for GTP hydrolysis was 17.5 min^{-1}, identical with that for ATP. The $S_{0.5}$ for GTP, however, was 750 μM, 17-fold higher than that for ATP. The GTPase activity exhibited positive cooperativity with respect to GTP concentration with a Hill coefficient of 2.0 at GTP concentrations near $S_{0.5}$ (350–1000 μM GTP, plot not shown).

Effect of GTP on RecA Protein Catalyzed Hydrolysis of ATP. The dependence of the initial rate of ATP hydrolysis on ATP concentration was measured in the presence of various fixed concentrations of GTP. The results are presented as a double-reciprocal plot in Figure 3. The curves intersect at

[2] On the basis of the binding stoichiometry of 1 recA monomer/4 nucleotides, 2 μM recA protein is sufficient to coat 30% of the ssDNA present (Bryant et al., 1985).

[3] $S_{0.5}$ is the substrate concentration required for half-maximal velocity. In the absence of cooperativity, the half-saturation point is equivalent to the Michaelis constant, K_m, but is more properly termed $S_{0.5}$ in cooperative systems (Neet, 1980).

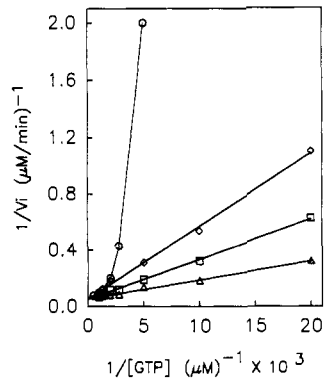


FIGURE 4: Effect of ATP on recA protein catalyzed hydrolysis of GTP. Reactions were carried as described under Experimental Procedures. Reaction solutions contained 30 μM ϕx ssDNA, 1.0 μM recA protein, the indicated concentration of [^{3}H]GTP, and either 0 μM ATP (circles), 50 μM ATP (triangles), 250 μM ATP (squares), or 500 μM ATP (diamonds). The points represent initial rates of GTP hydrolysis.

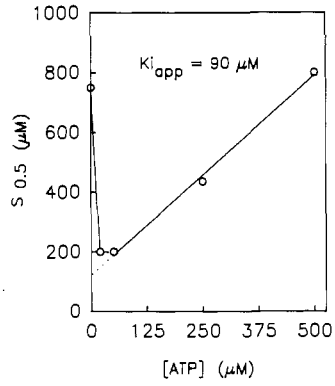


FIGURE 5: Dependence of $S_{0.5}$(GTP) on ATP concentration. GTP concentrations required for half-maximal GTP hydrolysis were determined from the data presented in Figure 3 and plotted versus ATP concentration. The extrapolated intercept of the linear region of the plot ([ATP] > 50 μM) on the [ATP] axis corresponds to a K_i of 90 μM for the competitive inhibition of GTP hydrolysis by ATP.

a common point on the $1/V_i$ axis, indicating that GTP acts as a competitive inhibitor of ATP hydrolysis. A replot of the $S_{0.5}$ for ATP versus GTP concentration yields a linear relationship and an apparent K_i of 150 μM for the competitive inhibition of ATP hydrolysis by GTP (plot not shown). Furthermore, in the presence of GTP (100–1000 μM) the Hill coefficient for ATP hydrolysis was reduced from 2.7 to 1.0 (plot not shown).

Effect of ATP on RecA Protein Catalyzed Hydrolysis of GTP. The dependence of the initial rate of GTP hydrolysis on GTP concentration was measured in the presence of various fixed concentrations of ATP. As shown in Figure 4, the turnover number for GTP hydrolysis was not affected by the presence of ATP. The $S_{0.5}$ for GTP, however, exhibited a complex dependence on ATP concentration (Figure 5). At low concentrations, ATP activated GTP hydrolysis by lowering the $S_{0.5}$ for GTP; in the presence of 50 μM ATP, the $S_{0.5}$ for GTP was reduced from 750 μM to 200 μM. At concentrations above 50 μM, ATP acted as a competitive inhibitor of the activated GTP hydrolysis reaction with an apparent K_i of 90 μM. When the ATP concentration required for optimal activation (50 μM) is taken into account, a K_i of 40 μM is obtained for the competitive inhibition of ATP-activated GTP hydrolysis by ATP. In addition, the Hill coefficient for GTP hydrolysis was reduced from 2.0 to 1.0 in the presence of ATP (plot not shown). Thus, ATP acts as both an activator and an inhibitor recA protein catalyzed GTP hydrolysis.

Effect of AMP-PNP on RecA Protein Catalyzed Hydrolysis of ATP and GTP. The effect of the nonhydrolyzable ATP analogue AMP-PNP on both ATP and GTP hydrolysis by recA protein was examined. AMP-PNP was a competitive inhibitor of ATP hydrolysis (K_i = 60 μM) and reduced the Hill coefficient for ATP hydrolysis from 2.7 to 1.0 (data not shown). In addition, AMP-PNP was found to activate GTP hydrolysis; at 200 μM GTP, the presence of 50 μM AMP-PNP resulted in a 4-fold increase in the rate of GTP hydrolysis (data not shown). These results indicate that hydrolysis of ATP is not required for activation of the recA protein nucleoside triphosphatase activity.

RecA Protein Catalyzed Hydrolysis of ATP and GTP at pH 6.2. The dependence of ATP hydrolysis and GTP hydrolysis on substrate concentration was also measured at pH 6.2 (Figure 2). At pH 6.2, ATP hydrolysis did not exhibit the cooperative dependence on ATP concentration that was seen at pH 7.5. This effect of pH on the kinetics of ATP hydrolysis has been noted previously (Weinstock et al., 1981a). GTP hydrolysis still exhibited positive cooperativity with respect to GTP concentration at pH 6.2, but the $S_{0.5}$ for GTP was 150 μM compared to 750 μM at pH 7.5. The turnover number for both ATP and GTP hydrolysis at pH 6.2 was 17 min^{-1}, similar to that measured at pH 7.5.

GTP as a Cofactor in RecA Protein Catalyzed Three-Strand Exchange Reaction. The three-strand exchange reaction was used to determine whether GTP can substitute for ATP as a high-energy cofactor in recA protein promoted DNA-pairing processes. In the three-strand exchange reaction, a circular ϕx ssDNA molecule and a homologous linear ϕx dsDNA molecule are recombined by the action of recA protein to form a nicked circular dsDNA molecule and a linear ssDNA molecule. This reaction is strongly stimulated by SSB, which is included in the assay mixture. The substrates and products of this reaction are easily monitored by agarose gel electrophoresis (Cox & Lehman, 1981).

At pH 7.5, the recA protein catalyzed strand exchange reaction proceeded to 90% completion within 30 min when ATP (1 mM) was included as a cofactor [gels not shown; see Cox and Lehman (1981)]. In contrast, no strand exchange was detected after 90 min when GTP (1 mM) was substituted for ATP, even when 50 μM ATP was also included as an activation supplement. Similar results were obtained at pH 6.2.

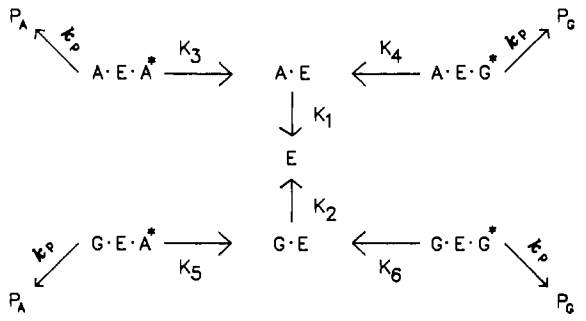
To explore the inability of GTP to support strand exchange, ATP hydrolysis and GTP hydrolysis were measured under strand exchange conditions. Strand exchange conditions differ from the conditions used for the kinetic measurements described above in that the recA protein is in excess relative to the ssDNA present (3.3 μM ssDNA, 2 μM recA). Therefore, maximal rates of ATP or GTP hydrolysis will be observed only if the ssDNA is completely coated by recA protein. ATP and GTP hydrolysis were measured both in the presence and absence of SSB.

At pH 7.5, the rate of ATP hydrolysis (1 mM ATP) was 11 μmol min^{-1} (μmol of recA protein)$^{-1}$ in the absence of SSB and increased to 19 μmol min^{-1} (μmol of recA protein)$^{-1}$ in the presence of SSB. In contrast, the rate of GTP hydrolysis (1 mM GTP) was only 4 μmol min^{-1} (μmol of recA protein)$^{-1}$ in the absence of SSB and *decreased* to <0.7 μmol min^{-1} (μmol of recA protein)$^{-1}$ in the presence of SSB. Thus, SSB stimulates ATP hydrolysis by the recA protein but suppresses GTP hydrolysis.

DISCUSSION

We have found that GTP will serve as a substrate for the

Scheme I



nucleoside triphosphatase activity of recA protein and that it is hydrolyzed with the same turnover number as ATP (17.5 min^{-1}).[4] Both ATP and GTP hydrolysis exhibit positive cooperativity with respect to substrate concentration, indicating that both activities are subject to substrate activation. The $S_{0.5}$ for GTP however, is 750 μM, 17-fold higher than the $S_{0.5}$ for ATP of 45 μM.

GTP acts as a competitive inhibitor of ATP hydrolysis, indicating that GTP can compete with ATP for the same NTP hydrolysis site on the recA protein. The K_i for GTP inhibition of ATP hydrolysis is 150 μM, considerably lower than the $S_{0.5}$ of 750 μM for GTP in the GTP hydrolysis reaction. The reason for this discrepancy became apparent when GTP hydrolysis was examined in the presence of ATP. Low concentrations of ATP were found to lower the $S_{0.5}$ for GTP; in the presence of 50 μM ATP, the $S_{0.5}$ for GTP is reduced from 750 μM to 200 μM. This lower $S_{0.5}$ is consistent with the K_i for GTP inhibition of ATP hydrolysis. ATP concentrations greater than 50 μM result in competitive inhibition of this ATP-activated GTPase activity with a K_i of 40 μM. This K_i is consistent with the $S_{0.5}$ for ATP in the ATP hydrolysis reaction. Additionally, the Hill coefficient for GTP hydrolysis is reduced from 2.0 to 1.0 in the presence of ATP, indicating that ATP can replace GTP as an activator of GTP hydrolysis. These results demonstrate that ATP can both activate and inhibit the hydrolysis of GTP by the recA protein. Apparently, binding of ATP is sufficient for activation of the GTPase activity since the nonhydrolyzable ATP analogue AMP-PNP also stimulates GTP hydrolysis by the recA protein.

The minimal kinetic model depicted in Scheme I was devised to account for the kinetic observations described above. In Scheme I, E refers to recA monomers that are contiguously bound to ssDNA. The cooperative binding of recA protein to ssDNA is strongly favored under the experimental conditions used in this study (Bryant et al., 1985). Therefore, recA protein–ssDNA binding equilibria are not included in this initial model. Also, for simplicity, this model is presented in terms of a single effector site and a single catalytic site.[5] This notation is not intended to imply that individual recA monomers have two NTP binding sites. In fact, the available evidence indicates that each recA monomer has only a single NTP binding site (Cox & Lehman, 1987). In Scheme I then, E could represent a dimer of strongly interacting monomeric recA subunits (one site each) or an oligomer of more weakly interacting recA subunits. We emphasize that the primary purpose of this initial model is to provide a simple kinetic scheme that is consistent with the kinetic effects reported in this paper.

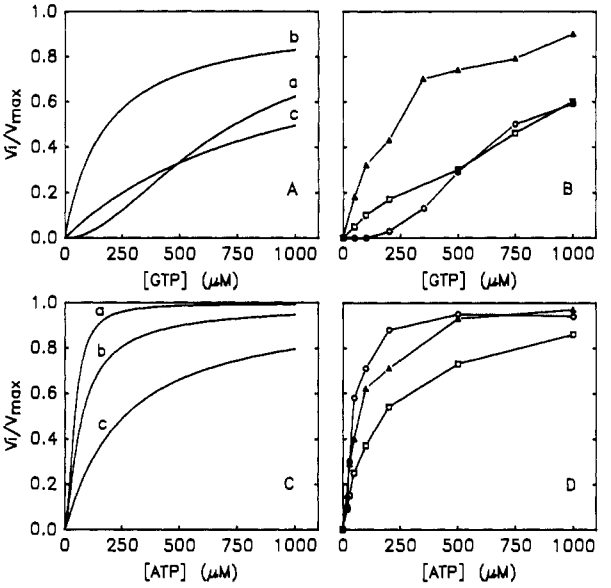


FIGURE 6: GTP and ATP hydrolysis curves predicted by Scheme I. GTP hydrolysis and ATP hydrolysis curves were calculated from eq 1 and 2, respectively, by using the parameters K_1 = 122 μM, K_2 = 23000 μM, K_3 = K_5 = 12 μM, and K_4 = K_6 = 23 μM. These parameters were chosen as follows. Pairs of values for K_2 and K_6 were obtained by setting eq 3 equal to the experimental value of 750 μM ([ATP] = 0). Values for K_1 and K_3 were similarly obtained by setting eq 4 equal to the experimental value of 45 μM ([GTP] = 0). Also, it was assumed that K_3 = K_5 and K_4 = K_6. Combinations of these parameters were selected to give calculated $S_{0.5}$(GTP) values ([ATP] = 50 or 500 μM) that approximated the experimental values. These combinations were then used to calculate full GTP hydrolysis and ATP hydrolysis velocity curves from eq 1 and 2. The experimental GTP hydrolysis and ATP hydrolysis curves were generated from initial velocity data (Figures 3 and 4, pH 7.5). (Panel A) Calculated GTP hydrolysis curves: (a) 0 μM ATP, (b) 50 μM ATP, and (c) 500 μM ATP. (Panel B) Experimental GTP hydrolysis curves: 0 μM ATP (circles), 50 μM ATP (triangles), and 500 μM ATP (squares). (Panel C) Calculated ATP hydrolysis curves: (a) 0 μM GTP, (b) 100 μM GTP, and (c) 500 μM GTP. (Panel D) Experimental ATP hydrolysis curve: 0 μM GTP (circles), 100 μM GTP (triangles), and 500 μM GTP (squares).

In Scheme I, ATP first binds to the effector site to form the complex A·E (dissociation constant K_1). A second ATP can then bind to the catalytic site to form the complex A·E·A* (dissociation constant K_3, $K_3 < K_1$) and is hydrolyzed to ADP and inorganic phosphate (rate constant k_p). This pathway is sufficient to account for the sigmoidal velocity curves that are obtained for ATP hydrolysis in the absence of GTP. Similarly, GTP can bind as an effector to form the complex G·E (dissociation constant K_2). A second GTP can then bind to the catalytic site to form the complex G·E·G* (dissociation constant K_6, $K_6 < K_2$) and is hydrolyzed to GDP and inorganic phosphate (rate constant k_p). When both ATP and GTP are present, two additional complexes must be considered. In one case, ATP binds to the effector site (dissociation constant K_1) and GTP binds to the catalytic site (dissociation constant K_4) to form a complex A·E·G* which leads to the hydrolysis of GTP. In the other case, GTP binds to the effector site (dissociation constant K_2) and ATP binds to the catalytic site (dissociation constant K_5) to form a complex G·E·A* which leads to the hydrolysis of ATP. The relative proportions of these complexes will depend on the concentrations of ATP and

[4] We have noted a pronounced dependence of the recA protein NTPase activity on temperature; at 1 mM GTP the rate of hydrolysis is <1 μmol min^{-1} (μmol of recA)$^{-1}$ at 26 °C (Menge, unpublished results) compared to 10.2 μmol min^{-1} (μmol of recA)$^{-1}$ at 37 °C. This temperature dependence may account for the low GTPase activity observed in an earlier study carried out at 30 °C (Weinstock et al., 1981b).

[5] Although the simple two-site model is sufficient for the purposes of the present discussion, a kinetic model employing one effector site and two catalytic sites may provide a better description of the ATP hydrolysis reaction kinetics (Hill coefficient = 2.7).

GTP as well as the values of the dissociation constants. The velocity equations that describe the hydrolysis of GTP and ATP were derived from Scheme I by using rapid-equilibrium assumptions (Segel, 1975) and are given:

$$\frac{V_i^{GTP}}{V_{max}^{GTP}} = \frac{\frac{[A][G]}{K_1K_4} + \frac{[G]^2}{K_2K_6}}{1 + \frac{[A]}{K_1} + \frac{[G]}{K_2} + \frac{[A][G]}{K_1K_4} + \frac{[A][G]}{K_2K_5} + \frac{[A]^2}{K_1K_3} + \frac{[G]^2}{K_2K_6}} \quad (1)$$

$$\frac{V_i^{ATP}}{V_{max}^{ATP}} = \frac{\frac{[A][G]}{K_2K_5} + \frac{[A]^2}{K_1K_3}}{1 + \frac{[A]}{K_1} + \frac{[G]}{K_2} + \frac{[A][G]}{K_1K_4} + \frac{[A][G]}{K_2K_5} + \frac{[A]^2}{K_1K_3} + \frac{[G]^2}{K_2K_6}} \quad (2)$$

Several relevant predictions can be made from Scheme I by examining the general equations for the dependence of the $S_{0.5}$ for GTP on ATP concentration and the dependence of the $S_{0.5}$ for ATP on GTP concentration. These equations were derived from eq 1 and 2, respectively, by setting V_i/V_{max} equal to 0.5 and are given:

$$S_{0.5}(GTP) = \left\{\left(1 + \frac{[A]}{K_5} - \frac{K_2[A]}{K_1K_4}\right)K_6 + \left[\left[\left(\frac{K_2[A]}{K_1K_4} - 1 - \frac{[A]}{K_5}\right)K_6\right]^2 + 4K_2K_6\left(\frac{[A]}{K_1} + \frac{[A]^2}{K_1K_3} + 1\right)\right]^{1/2}\right\} \Big/ 2 \quad (3)$$

$$S_{0.5}(ATP) = \left\{\left(1 + \frac{[G]}{K_4} - \frac{K_1[G]}{K_2K_5}\right)K_3 + \left[\left[\left(\frac{K_1[G]}{K_2K_5} - 1 - \frac{[G]}{K_4}\right)K_3\right]^2 + 4K_1K_3\left(\frac{[G]}{K_2} + \frac{[G]^2}{K_2K_6} + 1\right)\right]^{1/2}\right\} \Big/ 2 \quad (4)$$

Equation 3 shows that if $K_1K_4 < K_2K_5$, the $S_{0.5}$ for GTP will first decrease to a minimum and then increase in response to increasing concentrations of ATP. In contrast, eq 4 shows that $S_{0.5}$ for ATP will only increase in the presence of GTP if $K_1K_4 < K_2K_5$. Thus, in order for Scheme I to account for our experimental results, the overall equilibrium dissociation constant for A·E·G* (K_1K_4) must be less than the overall equilibrium dissociation constant for G·E·A* (K_2K_5).

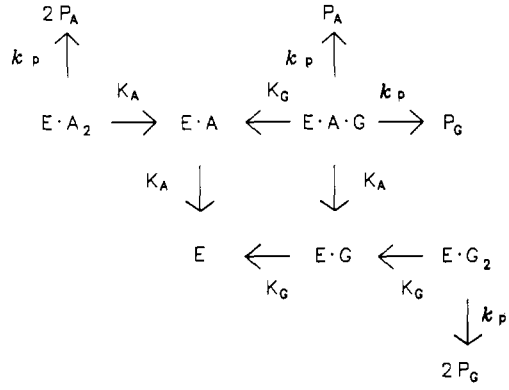
In the present study, the dissociation constants K_1 through K_6 are not known. However, by examining a range of parameters, it can be shown that Scheme I does predict patterns of velocity curves that are strikingly similar to those that were obtained experimentally. For example, Figure 6A shows simulated velocity curves for GTP hydrolysis that were calculated by using eq 1 and the parameters given in the figure legend. In the absence of ATP, the velocity curve is sigmoidal and the $S_{0.5}$ for GTP is 750 μM. With 50 μM ATP present, the velocity curve becomes hyperbolic (Hill coefficient = 1) and the $S_{0.5}$ for GTP is reduced to 200 μM. At 500 μM ATP, the velocity curve is still hyperbolic but the $S_{0.5}$ for GTP has increased to 1000 μM. These theoretical GTP hydrolysis curves are consistent with the experimental curves shown in Figure 6B. Figure 6C shows simulated velocity curves for ATP hydrolysis that were calculated by using eq 2 and the parameters that were used for the GTP hydrolysis curves. In the absence of GTP, the velocity curve is sigmoidal and the $S_{0.5}$ for ATP is 45 μM. With 100 μM GTP present, the velocity curve becomes less sigmoidal and the $S_{0.5}$ for ATP is increased to 70 μM. At 500 μM GTP, the $S_{0.5}$ for ATP is increased further to 250 μM. These theoretical ATP hydrolysis curves also match well with the experimental curves shown in Figure 6D.

The theoretical velocity curves shown in Figure 6 were calculated by using parameters in which $K_3 = K_5$ and $K_4 = K_6$. Velocity curves that are virtually identical with those in Figure 6 can also be obtained from Scheme I by using sets of parameters in which $K_3 < K_5$ and $K_4 < K_6$ (plots not shown). These two cases have distinctive mechanistic implications. If $K_3 = K_5$ and $K_4 = K_6$, then the affinity of the catalytic site for a given NTP will be the same whether ATP or GTP is acting as the effector. In this case, ATP and GTP will differ only in their affinity for the effector site. If $K_3 < K_5$ and $K_4 < K_6$, on the other hand, then the affinity of the catalytic site for a given NTP will be greater when ATP is the effector than when GTP is the effector. This would imply that ATP binding results in conformational changes in the recA protein that do not occur with GTP binding.

Independent evidence for an ATP-specific effect on the conformation of the recA protein was obtained when we attempted to substitute GTP for ATP as a high-energy cofactor for the recA protein catalyzed three-strand exchange reaction. Strand exchange was not detected in the presence of GTP, even when 50 μM ATP was included as an activation supplement. Furthermore, the GTPase activity of the recA protein that was apparent under standard NTPase assay conditions was eliminated under strand exchange conditions in the presence of SSB. In contrast, SSB stimulates recA protein promoted ATP hydrolysis under strand exchange conditions and allows ATP-dependent strand exchange to proceed efficiently.

The stimulatory effects of SSB on recA protein catalyzed ATP hydrolysis and ATP-dependent strand exchange have been the subject of intensive study. Cox and co-workers have proposed that both recA protein and SSB bind to ssDNA in stoichiometric amounts to form a unique ternary complex that is the reactive species in strand exchange (Monical et al., 1986). Kowalczykowski and co-workers, on the other hand, have suggested that SSB serves only to melt out secondary structure in the ssDNA before being displaced by recA protein and that the reactive complex in strand exchange consists only of ssDNA and stoichiometric amounts of recA protein (Kowalczykowski et al., 1987; Kowalczykowski & Krupp, 1987). Regardless of the exact mechanism of SSB stimulation, our results indicate that, in the presence of ATP, the recA protein adopts a conformation that is not displaced from ssDNA by SSB. GTP, on the other hand, apparently does not induce this conformation of the recA protein. Therefore, in the presence of GTP, recA protein may be displaced from ssDNA by SSB, and as a result GTP hydrolysis is eliminated. The ATP-specific conformational change indicated by the strand exchange experiments can be incorporated into Scheme I if $K_3 < K_5$ and $K_4 < K_6$.

Scheme II



Kowalczykowski has recently proposed an interesting model for the cooperative behavior of the recA protein ATPase activity (Kowalczykowski, 1986). By measuring the inhibition of azido-ATP cross-linking to recA protein by ATP, he determined a K_d of 2.5 μM for the binding of ATP to recA protein in the presence of ssDNA. The K_m for ATP in the ssDNA-dependent ATPase reaction, however, was 130 μM in his study. He concluded that a significant amount of ATP binding occurs at concentrations that are well below those required for stimulation of ATPase activity and therefore that the cooperative dependence of the ATPase activity on NTP concentration is not the result of cooperative binding of ATP. Instead, he proposed that the recA protein ATPase activity requires the formation of clusters of ssDNA-bound ATP–recA protein molecules and that an individual recA protein molecule becomes active in ATPase only when it is part of a cluster that has exceeded a certain minimal size (15 units). A model that is conceptually similar to the Kowalczykowski model is considered below in the context of the ATP and GTP hydrolysis kinetics that are described in this paper (Scheme II).

In Scheme II, E again refers to recA monomers that are contiguously bound to ssDNA. For simplicity, this model is presented in terms of two catalytic sites that have equal affinities for a given NTP and no cooperative interactions between them. Both catalytic sites must be occupied (with either ATP or GTP) before NTP hydrolysis can occur. The velocity equation for the hydrolysis of GTP according to Scheme II is given:

$$\frac{V_i^{GTP}}{V_{max}^{GTP}} = \frac{\dfrac{[A][G]}{2K_AK_G} + \dfrac{[G]^2}{K_G^2}}{1 + \dfrac{[G]}{K_G} + \dfrac{[A]}{K_A} + \dfrac{[A][G]}{K_AK_G} + \dfrac{[G]^2}{K_G^2} + \dfrac{[A]^2}{K_A^2}} \quad (5)$$

Scheme II does predict sigmoidal velocity curves for GTP hydrolysis in the absence of ATP and also predicts ATP stimulation of GTP hydrolysis at certain concentrations of ATP and GTP. However, Scheme II predicts that the $S_{0.5}$ for GTP will *only increase* in the presence of ATP, as shown:

$$S_{0.5}(GTP) = \frac{K_G + \sqrt{K_G^2 + 4K_G^2\left(1 + \dfrac{[A]}{K_A} + \dfrac{[A]^2}{K_A^2}\right)}}{2} \quad (6)$$

Therefore, Scheme II does not account for the decrease in the $S_{0.5}$ for GTP that is observed experimentally in the presence of ATP. A variation of Scheme II with cooperative interactions between the two catalytic sites also fails to account for a decrease in the $S_{0.5}$ for GTP in the presence of ATP (equations not shown). In Scheme II (cooperative or noncooperative), activation of GTP hydrolysis by ATP will require that ATP occupy one of the catalytic sites, which necessarily leads to an increase in the $S_{0.5}$ for GTP. In Scheme I, on the other hand, ATP can activate GTP hydrolysis without occupying a catalytic site and therefore ATP binding can lead to a reduction in the $S_{0.5}$ for GTP.

We emphasize that the kinetic models presented here are undoubtedly oversimplications and are intended only to identify general patterns of kinetic behavior. The kinetic results presented here (as interpreted according to Scheme I) and the ATP binding results of Kowalczykowski are consistent in the sense that both studies indicate that the binding of ATP to a recA–ssDNA complex is a necessary though not a sufficient requirement for ATP hydrolysis. The action of ATP as a positive effector of NTP hydrolysis (as described by Scheme I) and the ATP binding necessary to complete the formation of an active recA protein–ATP cluster of the requisite size (in the Kowalczykowski model) may, in fact, be equivalent mechanistic concepts. The precise molecular nature of the interaction of nucleoside triphosphates with the recA protein NTPase active site is under further investigation.

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Registry No. GTP, 86-01-1; ATP, 56-65-5; GTPase, 9059-32-9.

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