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Rapid Propagational Interactions of Slow Binding Inhibitor with RecA Protein Occur on the Longer Nucleoprotein Filaments

Jong-Il Kim

Department of Food and Microbial Technology, Seoul Women's University, Seoul 139-774, Republic of Korea

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RecA protein is a DNA-dependent ATPase. RecA protein-mediated ATP hydrolysis occurs throughout the filamentous nucleoprotein complexes of RecA and DNA. Nucleotide analog ATP[γS] may not act simply as a competitive inhibitor, leading to inhibition kinetic patterns that are informative. When a mixture of ATP and ATP[γS] is present at the beginning of reaction, a transient phase lasting several minutes is observed in which the system approaches the state characteristic of the new ATP/ATP[γS] ratio. This phase consists of a burst or lag in ATP hydrolysis, depending on whether ATP or ATP[γS] respectively, is added first. The transition phase reflects a slow conformational change in a RecA monomer or a general adjustment in the structure of RecA filaments. The RecA filaments formed on longer DNA cofactor were more sensitive, and respond more rapidly to ATP[γS] than on shorter DNA cofactors.

Keywords: adenosine-5′-O-(3-thiotriphosphate), cooperative binding, slow binding inhibitor, DNA-dependent ATPase, RecA protein

RecA protein, when bound to DNA, can catalyze the hydrolysis of ATP. Both single-stranded or double-stranded DNA (dsDNA) can serve to stimulate the hydrolysis of ATP by RecA protein. RecA protein binds cooperatively to DNA, forming a nucleoprotein filament. The filamentous structure contains one RecA monomer for every 3 nucleotides (Roca and Cox, 1990). The active species in ATP hydrolysis is a RecA filament, with the result that DNA oligonucleotides shorter than 60 nucleotides are poor cofactor. RecA protein-mediated ATP hydrolysis occurs throughout the filamentous nucleoprotein complexes of RecA and DNA. The turnover number for ATP is relatively low for an ATPase. The optimal k_{cat} on single-stranded DNA (ssDNA) approaches 30 min^{-1}. In addition to ATP, most naturally occurring rNTPs and dNTPs are hydrolyzed at some rate by RecA protein. In most cases, hydrolysis of another nucleotide occurs at a lower rate (Kowalczykowski, 1991). On dsDNA the optimal k_{cat} is about 30% lower, 21 to 25 min^{-1}. Reported Km for ATP generally range from 20 to 150 μM (Weinstock *et al*., 1981). In the absence of DNA free RecA protein binds to ATP, but hydrolysis seems not to occur under low salt conditions. Hydrolysis of ATP by RecA protein is a complex reaction with different characteristics depending on the DNA cofactor and pH. Under conditions used for DNA strand exchange, ATP hydrolysis is nearly completely DNA-dependent. ATP hydrolysis, in fact, has proven to be a reliable assay for DNA binding and can be the assay of choice because of the simple and accurate spectrophotometric method available for its measurement.

Both ATP and adenosine-5′-O-(3-thiotriphosphate)(ATP[γS]) bind reasonably tight to the free RecA protein of RecA protein bound to DNA with stoichiometry close to 1.0. The quantitative studies on the binding of nucleotides to the RecA protein demonstrated that there appears to be just one nucleotides binding site per RecA monomer. Many nucleotides act as competitive inhibitors of RecA protein-mediated ATP hydrolysis. The previous studies showed that ATP[γS] inhibits DNA-dependent ATP hydrolysis by RecA protein and inhibition of ATPase activity by ATP[γS] was competitive (Weinstock *et al*., 1981). Tight binding of ATP[γS] (K_d=0.5 μM) and a high degree of cooperativity in RecA protein binding were observed in the presence of modified DNA cofactor (εDNA) (Menetski and Kowalczykowski, 1985). The inhibitory effect of ADP was also investigated. The mode of inhibition for ADP involves the competitive and dissociative effect. As ATP hydrolysis progresses, ADP accumulates, which causes the ADP-mediated dissociation of RecA protein from DNA. All nucleoside triphosphates that are not hydrolyzed, as well as nucleoside diphosphates, reduce the affinity of the RecA protein for ssDNA. The binding of ATP to RecA protein in the presence of ssDNA is hyperbolic, while the hydrolysis rate dependence on ATP concentration is sigmoidal (Menetski and Kowalczykowski, 1985). This suggests that ATP binding itself is not sufficient to induce hydrolysis and may suggest some interactions between individual RecA protein molecules bound to DNA.

The mechanistic function of ATP binding and hydrolysis in RecA protein-mediated DNA strand exchange reaction is not entirely understood. Due to the complexity of these reactions, molecular interpretations have been difficult. In order to develop a mechanistic understanding of the role of ATP binding and hydrolysis, it is necessary to understand how RecA protein interacts with each of its substrates and how these interactions are affected by the presence of other substrates. However, the ATP hydrolytic reaction appears to be complicated. Binding of ATP is required but not sufficient to induce hydrolysis. It is

* For correspondence. E-mail: jikim@swu.ac.kr; Tel: +82-2-970-5638; Fax: +82-2-970-5977

difficult to separate the effect of ATP binding and hydrolysis on DNA-dependent RecA protein activity. Enzyme inhibitors have played an important part in elucidating kinetic mechanism of enzymatic reactions. By investigating the inhibition mechanism of RecA protein's DNA-dependent ATP hydrolytic reaction, the insight into interactions of ATP with RecA protein can be gained. Because binding and formation of RecA protein filament on DNA are required for ATP hydrolysis, inhibition can also result from a change of the DNA-binding properties of RecA protein. ATP analogs, such as ATP[γS], azidoATP and AMP-PNP are useful for this study. Numerous studies have previously demonstrated the usefulness of these ATP analog in studies with various ATP binding and ATP hydrolytic proteins (Abraham and Modak, 1988; Kowalczykowski, 1991). In this study, I sought to define in detail the interaction between ATP[γS] and RecA protein. Inhibition of DNA-dependent ATP hydrolysis by ATP[γS] is investigated by analyzing the transient kinetics of ATP[γS] inhibition.

Materials and Methods

RecA protein was purified by a procedure previously described (Cox *et al*., 1981). The concentration of RecA protein was determined by measuring the absorbance at 280 nm, using an extinction coefficient ε_{280}=0.59 A_{280} $mg^{-1}ml$. The purity of ATP and ATP[γS] was verified by thin layer chromatography. Concentrations of ATP and ATP[γS] were determined by measuring the absorbance at 259 nm, using an extinction coefficient of ε_{259}=15,400 $M^{-1}cm^{-1}$. Poly(dT) was purchased from Sigma. The length of poly(dT) was estimated by labeling the 5' end of poly(dT) as described. The length of the poly(dT) was heterogeneous, ranged from 340 to 1,000 nucleotides, and the average length was about 650 nucleotides. Oligo(deoxythymidylic acid), 60 bases in length$(dT)_{60}$, was obtained from GenScript, USA. The concentrations of poly, oligo(dT) were determined by absorbance at 264 nm, using an extinction coefficient of ε_{264}=8,520 $M^{-1}cm^{-1}$ and DNA concentrations are given in terms of nucleotides.

ATPase assay

The DNA-dependent ATPase activity of RecA protein was measured in a reaction mixture containing 25 mM Tris-acetate (pH 7.5), 5% glycerol, 10 mM $Mg(acetate)_2$, 1 mM DTT, 1 mM ATP, and ATP regenerating system (2.4 U/ml pyruvate kinase, 2.4 mM phosphoenol-pyruvate, 0.44 mM KCl). The final concentration of RecA protein was 2 μM and the concentration of DNA was 5 μM. All reactions were carried out at 37°C. Reactions were carried out in a volume of 0.5 ml in 0.5 cm path length, self-masking quartz cuvette. Unless otherwise indicated, reaction was initiated by addition of RecA protein after all other components were incubated at 37°C for 10 min. In an experiment a cuvette was in a thermojacketed holder in a spectrophotometer. Reaction progress was measured by monitoring ATP hydrolysis which is coupled to the ATP regenerating system. Regeneration of ATP from ADP and phosphoenolpyruvate is coupled with the oxidation of NADH to NAD^+, which can be monitored by a decrease in absorbance at 380 nm. Due to the high concentration of NADH (3 mM) used in this experiments, absorbances measured at 380 nm, so as to remain in the linear region of the spectrophotometer. High concentrations of NADH were necessary to ensure that a steady-state was reached under all conditions. Adding more regeneration enzymes does not increase the rate, so coupling system is never limiting the observed rate of ATP hydrolysis. ATP is constantly regenerated in this system so that no product (ADP) accumulation occurs. A system lag due to the coupling system was approximately 0.5 min. Rates of ATP hydrolysis were calculated from ΔA_{380} min^{-1} obtained at steady-state, using an extinction coefficient of 1,210 $M^{-1}cm^{-1}$ at 380 nm for NADH. Absorbances were continuously measured over a period of 1 h.

Results

Characterization of inhibition

High concentrations of ATP[γS] (on the order of 1 mM or more) have been shown to produce a rapid an tight inhibition of RecA protein-mediated ATP hydrolysis. In these experiments, I focused on inhibition patterns observed when concentrations of ATP[γS] were employed that were closer to the reported Kd for this inhibitor. Reaction progress curves reveal that inhibition develops slowly when ATP[γS] was added into a DNA-dependent ATP hydrolytic reaction promoted by RecA protein, as illustrated in Fig. 1. The relatively rapid initial velocities decrease slowly over several minutes to the steady-state velocities. The slow establishment is not due to enzyme inactivation or substrate depletion. These curves are characterized by initial velocities that are identical with control velocities established in the absence of inhibitor and final steady-state velocities that are significantly decreased relative to control. If the RecA protein is preincubated with ATP[γS] and the reaction monitored after adding the substrate ATP, there is slow release of inhibition and ultimately a steady-state rate is reached. For the transient phase, burst progressive curves were observed when ATP[γS] was added after ATP [Fig. 1(A)], and a lag was observed when ATP[γS] was added prior to ATP [Fig. 1(B)]. In the experiment from Fig. 1, a slow reduction in ATP hydrolysis reflects the association of

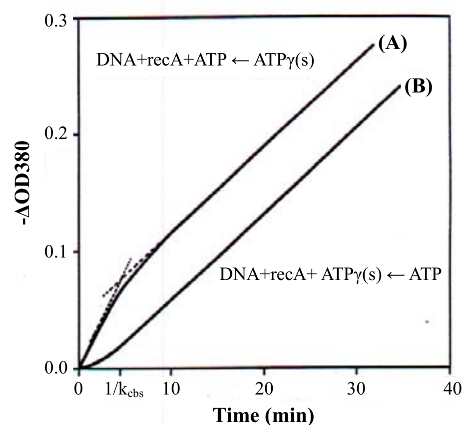


Fig. 1. The Effects of ATP[γS] on poly(dT)-dependent ATP hydrolysis. Effect of order of addition of nucleotides. The poly(dT)-dependent reactions were monitored with the spectrophotometic coupled assay. Reactiion mixture contained 5 μM poly(dT), 2 μM RecA protein, 1 mM ATP and 5 μM ATP[γS] at pH 7.5. RecA protein was preincubateed with poly(dT) for 5 min, ATP was added, and incubation was continued for 10 min; then was added in experiment indicated by (A). As plotted, t=0 is the point at which ATP[γS] was added. (B) RecA protein and poly(dT) were incubated for 5 min, ATP[γS] was added, incubation was continued for 10 min, and then the reaction was initiated with ATP at t=0.

ATP[γS] with RecA protein, and that RecA protein-ATP[γS] complexes accumulate during transient phase. The two steady-state rates were identical. Inhibitors producing these sorts of progress curves have been known as slow-binding inhibitors (Waley, 1993).

The slow establishment of the full inhibition can occur as a result from a slow establishment of interaction of RecA protein with a competitive inhibitor. Any burst (or lag) in substrate utilization that is followed by a linear accumulation of product can be described by the expression

$$V_t = V_f + (V_i - V_f)\exp(-k_{obs}t) \quad (1)$$

$$P_t = V_f + (V_i - V_f)[1-\exp(-k_{obs}t)] / k_{obs} \quad (2)$$

where V_t is the velocity as a function of time, V_i is the initial velocity before adding ATP[γS], V_f is the final(linear) steady-state velocity after the burst or lag, k_{obs} is the apparent first-order rate constant for the approach to steady state, and P is product concentration. The tangent to curve, as t goes to zero, is given by the expression $P=V_it$, while the relationship $P=V_i t+(V_i - V_f)/k_{obs}$ defines the asymptote of the curve as t tends to infinity. At the intersection point of these two linear curves, $t=1/k_{obs}$. Equation 1 is a general expression and describes any mechanism of inhibition in which the steady state is reached by a first-order process.

Effect of inhibitor concentration on the transition rates

In order to determine the possible inhibition mechanism of RecA protein-mediated ATP hydrolysis by ATP[γS], transient kinetics relating ATP hydrolysis to ATP[γS] concentrations were analyzed. Values of variables affected by ATP[γS] concentration were measured from a time course of ATP hydrolytic reactions where ATP[γS] was added after initial ATP hydrolysis had been established in steady-state (Fig. 2). Steady-state rates (Vf) were taken from the linear region of the curve established after burst. When 3 μM ATP[γS] was added to a poly(dT)-dependent reaction where the ATP concentration was 1 mM, 55% initial hydrolytic rate remained in the final steady-state. Twenty micromolar ATP[γS] was enough to repress ATP hydrolysis to nearly 5% of its original value. The rate of ATP hydrolysis in the final steady-state is dependent on the ratio of ATP and ATP[γS] concentration in the reaction mixture (Fig. 3). In reactions with higher ATP concentration, more ATP[γS] was needed to obtain the same extent of inhibition as the reactions where less ATP is present. This indicates ATP[γS] can act as a reversible, competitive inhibitor.

Effect of DNA length on the response to inhibitor

The effect of DNA length was investigated to distinguish the several possible causes of the transition phase. Transient phase reflects there exists slow process in response to ATP[γS]. If the slow step is some process propagated lengthwise through the filament, the length of transition phase should be proportional to the length of transition phase should be proportional to the length of the filament. If the slow step is conformational change or exchange of bound ligand occurring simultaneously throughout the filament, then the length of the transient phase should be unaffected by filament length. Both effects of the sensitivity of ATP hydrolytic reaction to ATP[γS] and the length of transition phase as a function of DNA length were examined. The size of the DNA dictates the length of the RecA nucleoprotein filament.

Binding of ATP[γS] to the RecA-DNA complex is highly cooperative under various conditions. The effect of inhibition by ATP[γS] on the kinetics of ATP hydrolysis could be strongly influenced by cooperative interactions between RecA monomers within the RecA nucleoprotein filament. The effectiveness of cooperative interaction depends on the length of RecA filament. In order to determine the effect of DNA size on the response to ATP[γS] inhibition, shorter dT oligomers (dT_{60}, dT_{30}, dT_{18}) were used as well as polydT. Oligomer $(dT)_{60}$ is long enough to activate the ATPase as effectively as poly(dT) at pH 7.5

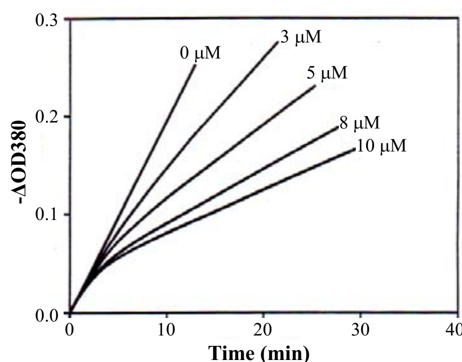


Fig. 2. Progress curves for inhibition of RecA protein-medated ATP hydrolysis at pH 7.5. RecA protein (2 μM) was preincubated with 5 μM poly(dT) for 5 min, 1 mM ATP was added, and incubation was contiued for 10 min; then ATP[γS] was added at concentrations of 3, 5, 8, and 10 μM.

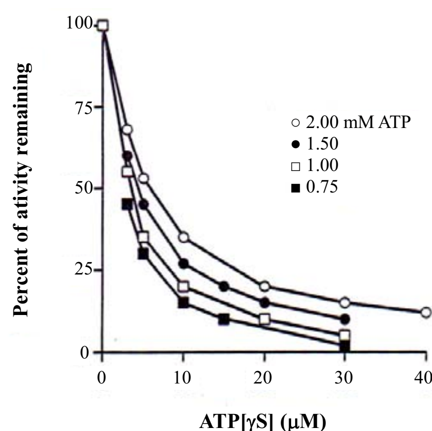


Fig. 3. The extent of ATP[γS] inhibition of poly(dT)-dependent ATP hydrolysis at pH 7.5. Concentrations of ATP were 2 mM (○), 1.5 mM (●), 1 mM (□), and 0.75 mM (■) was added after preincubation of RecA protein (2 μM) and 5 μM poly(dT) for 5 min. After 10 min additional incubation, ATP[γS] was added. Data reflect the fractions between steady-state rates of ATP hydrolysis in the reactions with indicated concentrations of ATP[γS] and rate of ATP hydrolysis in reaction without ATP[γS], which reflect the initial rate of the reaction with ATP[γS].

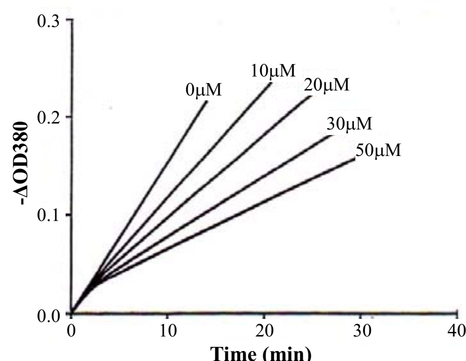


Fig. 4. The Effects of ATP[γS] on $(dT)_{60}$-dependent ATP hydrolysis. Time course of $(dT)_{60}$-dependent ATP hydrolysis in the presence of ATP[γS] at pH 7.5. RecA protein (2 μM) was preincubated with 5 μM (dT) 60 for 5 min, 1 mM ATP was added, and incubation was continued for 10 min; then ATP[γS] was added at concentrations of 10, 20, 30, and 50 μM.

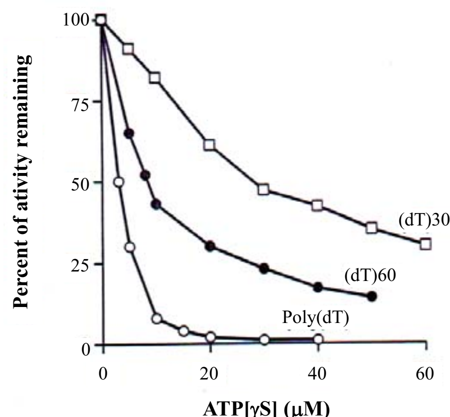


Fig. 6. The extent of ATP[γS] inhibition at pH 6.5. ATP hydrolytic reactions contained RecA protein (2 μM), 5 μM DNA ,1 mM ATP was added, and incubation was continued for 10 min; then ATP[γS] was added; $(dT)_{30}$-dependent reaction (□), $(dT)_{60}$-dependent reaction (●), and poly(dT)-dependent reaction (○).

(Brenner *et al*., 1987). The rate of ATP hydrolysis in reaction with $(dT)_{60}$ also decreased as increasing amounts of ATP[γS] were added (Fig. 4). The final steady-state rates of two reactions were compared in Fig. 5. Reactions with poly(dT) were more sensitive to ATP[γS] inhibition than reactions with $(dT)_{60}$ at pH 7.5. This phenomenon is more evident at pH 6.5 (Fig. 6).

There was a gradual increase (a shorter lag) in the measured transition rate as ATP[γS] concentration increased. The transition rates (k_{obs}) corresponded to the reciprocal of the time point at the intersection of the asymptotic lines defined by V_i and V_f (see Fig. 1). The plot of ATP[γS] concentration versus transition rate is nonlinear (Fig. 7). This suggests that the mechanism of inhibition by ATP[γS] apparently follows Scheme II in discussion. The apparent association rate of ATP[γS] with RecA protein on DNA was estimated to be 3.5×10^4 M/min for poly(dT)-dependent reaction, and 1.6×10^4 M/min for $(dT)_{60}$-dependent reaction at pH 7.5 in the range of 10 μM ATP[γS]. Transition rates were somewhat faster with poly(dT) than with $(dT)_{60}$, but differences were not large in relation to the difference in DNA length. This suggests that the slow step that leads to the transition phase is a conformational change or slow binding occurring throughout the filaments.

The effect of ATP[γS] concentration on the transition phase was also examined in lag type kinetics, where preincubation of RecA protein with ATP[γS] and DNA was followed by addition of ATP (1 mM). In this experiment dissociation of ATP[γS] (and/or substitution of ATP) is the concern of analysis. The more ATP[γS] was present in the preincubation mixture, the longer time was required for relieving inhibition and reaching to new steady-state. The association of ATP with the RecA filament which was prebound by ATP[γS] was more rapid in $(dT)_{60}$-dependent reaction than in reaction with poly(dT). This indicates that the length of the transient phase depends upon the length of the individual filament and that a cooperative effect on ATP[γS] from others that may be more

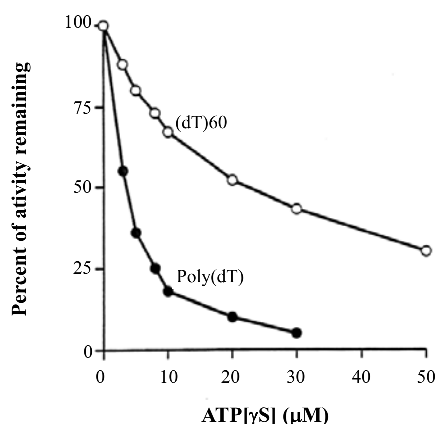


Fig. 5. The extent of ATP[γS] inhibition at pH 7.5. ATP hydrolytic reactions contained RecA protein (2 μM), 5 μM DNA, 1 mM ATP was added, and incubation was continued for 10 min; then ATP[γS] was added.

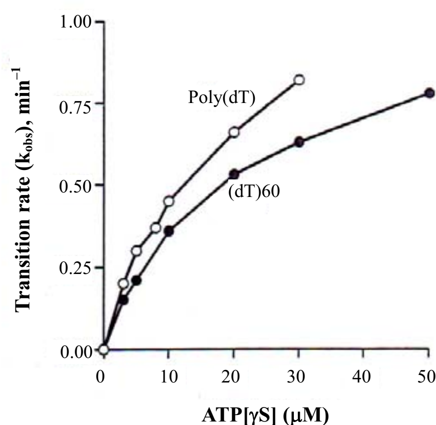


Fig. 7. The effect of ATP[γS] concentration on the transition rate in burst type kinetics at pH 7.5. ATP hydrolytic reactions contained RecA protein (2 μM), 5 μM DNA, 1 mM ATP was added, and incubation was continued for 10 min; then ATP[γS] was added.

than 60 nucleotides (20 monomers) distant. Lag type kinetics have not been more considered. The burst type kinetics were considered for the analysis of inhibition mechanism, since binding of ATP[γS] can be evaluated in burst kinetics. It seems that the slow process is to be mediated longitudinally through the filament in both type of kinetics. However, the effect of DNA length was reversed in the two type kinetics.

pH effect

Oligomers of dT shorter than 30 base long appeared ineffective as DNA cofactors for RecA protein ATPase activity. The observation that short DNA oligomers were less effective as cofactors for ATP hydrolysis than longer oligomers at pH 7.5 could indicate weaker binding to RecA protein. This suggests the apparent affinity of RecA protein for oligonucleotides is a strong function of the oligonucleotide length. The apparent dissociation constants reported are 1.8 μM for poly(dT), 5.6 μM for $(dT)_{50}$, 63 μM for $(dT)_{30}$, and 333 μM for $(dT)_{25}$ at pH 7.2 (Brenner *et al*., 1987). However, comparable amounts of ATP hydrolysis were observed in reaction with $(dT)_{30}$ and $(dT)_{18}$ at pH 6.5, and a measurable increase of ATP hydrolysis rate was observed with decreasing pH. Lowering the pH seems to stimulate RecA protein binding to shorter oligonucleotids. Observed rates and cooperativity of ATP hydrolysis with different length of DNA cofactor at various pHs are summarized in Table 1.

The extent and transition rate of ATP[γS] inhibition of ATP hydrolysis reactions with various length of homopolymeric (dT) were also examined at pH 6.5 (Figs. 6 and 8). In poly (dT) and $(dT)_{60}$-dependent reaction, the extent and transition rate for the inhibition always appeared higher at pH 6.5 than at pH 7.5 (Figs. 5 and 6). Reactions with $(dT)_{18}$, on which RecA protein forms filament with one helical turn, was insensitive to ATP[γS] inhibition up to 100 μM ATP[γS]. The RecA nucleoprotein filaments formed on longer DNA exhibited more rapid response to ATP[γS] inhibition. Inhibition constants (Ki_{app}) for ATP hydrolysis were also measured from Dixon type analysis at pH 7.5, which were 0.5 μM for reactions with poly(dT), 4 μM for $(dT)_{60}$, and 30 μM (at pH 6.5) for $(dT)_{30}$. This demonstrates that ATP[γS] has higher affinity for longer RecA nucleoprotein filament than shorter one, and suggests that may be a rate-limiting nucleation step that is facilitated by longer filaments with more potential nucleation sites.

Table 1. Kinetic properties for DNA-dependent ATP hydrolysis and ATP[γS] inhibition

DNA cofactor	$(dT)_{18}$	$(dT)_{30}$	$(dT)_{60}$	Poly(dT)
Rate of ATP hydrolysis (μM/min)				
pH 6.5	10	30	39	44
pH 7.5		4	30	40
Hill coefficient for ATP hydrolysis				
pH 6.5		1.4	2.5±0.1	3.0±0.1
pH 7.5			2.3±0.3	2.9±0.2
Ki_{app} for ATP[γS] at pH 6.5		30 μM	4 μM	0.5 μM

Reaction conditions for ATP hydrolysis contained 5 μM DNA, 2 μM RecA protein and 1 mM ATP

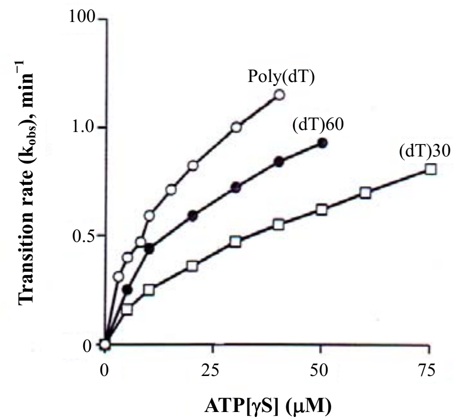


Fig. 8. The effect of ATP[γS] concentration on the transition rate in burst type kinetics at pH 6.5. $(dT)_{30}$-dependent reaction (□), $(dT)_{60}$-dependent reaction (●), and poly(dT)-dependent reaction (○).

Discussion

When judged from the kinetic parameters, ATP[γS] binds more tightly to RecA protein than ATP under most conditions. The interaction of ATP[γS] with RecA nucleoprotein filaments showed a time-dependent hysteretic behavior, where response is dependent on the concentration of ATP[γS]. The stable RecA protein-ATP[γS] complexes inhibit the ATPase activity of RecA protein. Both binding and dissociation of ATP[γS] are slow relative to the turn-over of ATP in the normal hydrolytic reaction. DNA length has a great effect on the response of RecA protein to inhibitor. The RecA filaments formed on longer DNA cofactor were more sensitive, and respond more rapidly to ATP[γS] than on shorter DNA cofactors. 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 slow rate of dissociation and the fact that the rate of formation of these complexes is slower than the rate of turnover of ATP during hydrolysis.

When ATP[γS] is in excess to RecA protein it can act as a simple competitive inhibitor. For competitive inhibitors, Scheme I or II can be considered. In the scheme of inhibition mechanism I, k_{on} is the second-order rate constant for formation of RI, the complex of inhibitor and enzyme, and k_{off} is the first-order rate constant for decomposition of this complex. K_i, the dissociation constant for RI, is the ratio of k_{off}/k_{on}.

Scheme I

k_{off} ↕ $k_{on}[I]$

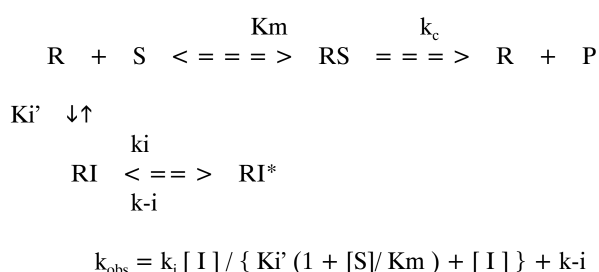
RI

$$k_{obs} = k_{on}[I] / (1 + [S]/Km) + k_{off} \quad (3)$$

According to this simple mechanism of inhibition, the observed rate constant for the approach to steady-state, is

given by equation 3 and predicts a plot of k_{obs} vs [1]/(1+[S]/Km) will be linear with slope and intercept of k_{off} and k_{on}, respectively. This mechanism assumes that the stable, enzyme-inhibitor complex, RI, forms directly from a bimolecular combination of RecA protein and ATP[γS]. With this mechanism, it is assumed that the interaction of competitive inhibitor with the enzyme is slow because its concentration is so low and/or it encounters barriers to its binding at the site of substrate interaction. As the strength of interaction between an enzyme and a tight-binding inhibitor increases, a stage is reached when the equilibrium of the reaction can not be established rapidly. The apparent first-order rate constant (k_{on}[I]) will be small when only a very low concentration of inhibitor is required to demonstrate the inhibition.

Scheme II



$$k_{obs} = k_i [\,I\,] / \{ Ki' (1 + [S]/Km) + [\,I\,] \} + k\text{-}i$$

For Scheme II, it is assumed that there is an initial rapid interaction between enzyme and inhibitor to form RI which then undergoes a slow isomerization reaction to RI*. Binding of inhibitor itself is not sufficient for the inhibition. The apparent first rate constant (k_{obs}) varies as a hyperbolic function of inhibitor concentration with scheme II, but as a linear function in scheme I. Results shown in Fig. 5, suggest that ATP[γS] inhibition follows Scheme II.

Binding of ATP[γS] to the active site of RecA monomer has two effects; it blocks the binding of ATP to that monomer, but stabilizes high affinity conformation in adjacent subunits through cooperative protein-protein interaction. Nucleotide-mediated changes in the domain structure of the RecA protein of *Escherichia coli* were demonstrated (Kobayashi *et al*., 1987). Trypsin digestion as a means of investigating changes in the structural properties of RecA protein accompanying the binding of different nucleoside triphosphates could explain the conformational changes of RecA protein complexed with dATP, ATP, or the ATP analogue (ATP[γS]). Interactions of *E. coli* RecA protein with ATP and its analogues were also studied by circular dichroic (CD) spectroscopy (Watanabe *et al*., 1994). A non-hydrolyzable ATP analogue, (ATP[γS]), gave a spectral change of CD band at around 260 nm.

The differences in the transition rate of ATP[γS] inhibition by RecA protein in the presence of long versus short DNA molecules reflects the effects of cooperativity on the transition rates of response to inhibitor by enzymes activated by polymeric cofactors that provide multiple binding sites for the enzymes. Nearest-neighbor interaction of two bound RecA proteins could have a great effect on the response of protein to ligand. Inhibitor binding might cause a conformational change in a RecA protein monomer bound to DNA. Once this isomerization is nucleated in one DNA site, the effect may be rapidly propagated longitudinally through DNA. The transient phases reflect conformational changes that are propagated longitudinally through the filament. Nearest-neighbor interaction of two bound RecA proteins could facilitate the rapid transmission of slow conformational change through RecA filament. The slow conformation change occurring in monomer is communicated to its neighbor via cooperative interactions.

Acknowledgements

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