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# Alteration of the Nucleoside Triphosphate (NTP) Catalytic Domain within *Escherichia coli* recA Protein Attenuates NTP Hydrolysis but Not Joint Molecule Formation*

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**The hydrolysis of the nucleoside triphosphates, such as ATP or GTP, plays a central role in a variety of biochemical processes; but, in most cases, the specific mechanism of energy transduction is unclear. DNA strand exchange promoted by the *Escherichia coli* recA protein is normally associated with ATP hydrolysis. However, we advanced the idea that the observed ATP hydrolysis is not obligatorily linked to the exchange of DNA strands (Menetski, J. P., Bear, D. G., and Kowalczykowski, S. C. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 21–25); instead, ATP binding resulting in an allosteric transition to an active form of the recA protein is sufficient. In this paper, we extend this conclusion by introducing a mutation within a highly conserved region of the recA protein that, on the basis of sequence similarity, is proposed to interact with the pyrophosphate moiety of a bound NTP molecule. The conservative substitution of an arginine for the invariant lysine at position 72 reduces NTP hydrolysis by ≈600–850-fold. This mutation does not significantly alter the capacity of the mutant recA(K72R) protein either to bind nucleotide cofactors and single-stranded DNA or to respond allosterically to nucleotide cofactor binding. Despite the dramatic attenuation in NTP hydrolysis, the recA(K72R) protein retains the ability to promote homologous pairing and extensive exchange of DNA strands (up to 1.5 kilobase pairs). These results both identify a component of the catalytic domain for NTP hydrolysis and demonstrate that the recA protein-promoted pairing and exchange of DNA strands mechanistically require the allosteric transition induced by NTP cofactor binding, but not the energy educed from NTP hydrolysis.**

In promoting the homologous pairing and subsequent exchange of DNA strands *in vitro*, the *Escherichia coli* recA protein has become the prototype for investigating biochemical events that underlie genetic recombination (2). Fundamental to all activities of the recA protein is the capacity to interact with DNA and nucleotide cofactors. Unlike other DNA-binding proteins, the stability (3) and structure (4, 5) of recA protein-DNA complexes is modulated by nucleotide cofactors; the binding of either ATP or dATP allosterically induces the high affinity DNA binding state of the recA protein with its associated extended nucleoprotein filament structure that is essential to all recA protein-promoted activities, whereas the absence of cofactor or the binding of ADP produces a low affinity binding state accompanied by a compact nonfunctional filament structure (6). Consequently, the DNA-dependent ATP hydrolysis activity of the recA protein is proposed to both regulate and facilitate recA protein binding to and dissociation from DNA (3, 6). ATP hydrolysis, however, is not essential for the exchange of DNA strands since extensive strand exchange is promoted by the recA protein when the essentially nonhydrolyzable analogue ATPγS[1] is substituted for ATP (1, 7). These results argue for a mechanism of energy transduction that relies on the free energy of ATP binding to allosterically induce the active form of the recA protein and ATP hydrolysis serving to unidirectionally complete the cycle; hence, ATP hydrolysis *per se* is not crucial to the exchange of DNA strands. The importance of this mechanistic issue has been elevated with the isolation of DNA pairing activities from eukaryotic sources that have no requirement for NTP hydrolysis (8–13).

The utilization of ATP or dATP by the recA protein bears a mechanistic resemblance to that of both translation elongation factor Tu and the signal-transducing ras proteins of the G-protein family in that NTP hydrolysis serves as a molecular switch that allows these proteins to convert between active (NTP-bound) and inactive (NDP-bound) conformations. Structural determination of the adenylate kinase (13, 14), ras p21 (15, 16), and elongation factor Tu (17) proteins and, very recently, the recA protein (18, 19) reveals that each contains a nucleotide-binding fold comprised in part by a conserved phosphate-binding loop. Furthermore, a comparison (16) between structures of the p21 ras protein in the presence GDP (15) and a GTP analogue (16) reveals that the "molecular switch" in the protein conformation is predominantly induced by the alteration of interactions between the phosphate-binding loop and the hydrolyzed γ-phosphate. This phosphate-binding loop motif, with the consensus sequence (G/A)$X_4$GK(T/S), was originally noted by Walker *et al.* (20)

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[1] The abbreviations used are: ATPγS, adenosine 5′-γ-(thiotriphosphate); ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, single-stranded DNA-binding; εM13 ssDNA, modified M13 ssDNA containing 1,$N^6$-ethenoadenosine and 3,$N^4$-ethenocytidine residues; RFI, relative fluorescence increase; STMP, salt titration midpoint.

as a sequence element ("A" site) prevalent in a number of purine nucleotide-binding proteins, including the recA protein. This A site motif within the nucleotide-binding fold of a number of diverse proteins (RAD 3 (21), rho (22), uvrA (23), and uvrB (24, 25)) is involved in catalysis since alteration of the invariant lysine residue results in nonfunctional mutant proteins that have a reduced ability to hydrolyze ATP, but still bind ATP.

To address further the issue of energy transduction by the recA protein in the pairing and exchange of DNA strands and to extend our understanding of the mechanistic significance of the molecular switch between active and inactive conformations of the recA protein, we sought to create a mutant recA protein that is deficient exclusively in NTP hydrolysis, but proficient in homologous pairing and exchange of DNA strands. The mutation (lysine 72 to arginine) was constructed within the putative A site of the recA protein ($^{66}$GPESSGKT$^{73}$) and is conservative with respect to size and charge. The resultant mutant protein, recA(K72R), is greatly attenuated in NTP hydrolysis activity, yet retains the ability to bind both DNA and nucleotide cofactor and to respond allosterically to nucleotide cofactor binding. Most significantly and in contrast to previous studies involving mutagenesis within the putative A sites of other proteins, this mutant recA protein remains functionally proficient in promoting the homologous pairing and exchange of DNA strands. These results demonstrate that the allosteric transition induced by NTP cofactor binding, but not the energy derived from NTP hydrolysis, is essential in the pairing and exchange of DNA strands by the recA protein.

## MATERIALS AND METHODS

*Reagents*—Chemicals were reagent-grade, and solutions were prepared using Barnstead NANOpure water. ADP, dADP, and dATP was purchased from Sigma; ATP and ATPγS were from Pharmacia LKB Biotechnology Inc. and Boehringer Mannheim, respectively. All nucleotide cofactors were dissolved as concentrated stock solutions at pH 7.5, and their concentrations were determined spectrophotometrically using an extinction coefficient of $1.54 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 260 nm.

*Proteins*—The *recA2201* allele encoding the recA(K72R) protein consists of a single point mutation (AAA → AGA) created using oligonucleotide site-directed mutagenesis (26) and verified by dideoxy sequencing the entire *recA2201* gene. The wild-type *recA* gene, cloned into M13mp18 using *Sph*I and *Kpn*I restriction sites, was obtained from A. John Clark (University of California, Berkeley, CA). The *recA2201* gene was cloned into the expression vector pKK223-3 (Pharmacia) under control of the *tac* promoter using unique restriction sites engineered (26) upstream (*Xma*I) and downstream (*Hin*dIII) of the recA coding region.[2] The recA(K72R) protein was expressed in *E. coli* strain JC10287 (Δ*recA*) (27) and was purified using a modified Polymin P preparative procedure (28). The wild-type recA protein was purified from *E. coli* strain JC12772 (27) using a preparative protocol[3] based on spermidine acetate precipitation (29). recA protein concentration was determined using an extinction coefficient of $2.7 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 280 nm. *E. coli* SSB protein was purified from strain RLM727 as described (30), and its concentration was determined using an extinction coefficient (31) at 280 nm of $3.0 \times 10^4$ M$^{-1}$ cm$^{-1}$. Phosphoenolpyruvate and pyruvate kinase were both purchased from Sigma.

*DNA*—Single- and double-stranded DNAs were prepared from bacteriophage M13mp7 using the procedure described by Messing (32). The duplex DNA was linearized by digestion with *Eco*RI restriction endonuclease. Molar nucleotide concentrations were determined using extinction coefficients of 6500 M$^{-1}$ cm$^{-1}$ for duplex DNA and 8784 M$^{-1}$ cm$^{-1}$ for single-stranded DNA at 260 nm. εM13 DNA was prepared from the phage DNA as described (3), whereas poly(dT) was purchased from Pharmacia; respective concentrations were determined using extinction coefficients of 7000 (33) and 8520 M$^{-1}$ cm$^{-1}$ at 260 nm.

*DNA Binding Assays*—The binding of the recA protein to εM13 ssDNA and the stability of the resultant complexes to dissociation by NaCl were monitored fluorometrically as previously described (3). The concentration of nucleotide cofactors was 1 mM, with the exception of ATPγS, which was 500 μM. A regenerating system consisting of 10 units of pyruvate kinase/ml and 3 mM phosphoenolpyruvate was present in all reactions that contained ATP or dATP cofactors. The experiments were carried out in buffer consisting of 25 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, and 0.1 mM dithiothreitol at 25 °C with continuous stirring.

*Nucleotide Cofactor Binding and Hydrolysis*—The binding of ATP, dATP, or ATPγS to recA protein-εM13 ssDNA complexes was monitored fluorometrically using DNA binding assay conditions, with the exception that the regenerating system used in the dATP experiments contained 30 units of pyruvate kinase/ml.

The DNA-dependent or high salt-induced hydrolysis (34) of ATP or dATP promoted by the wild-type recA protein was measured as described previously (35). The hydrolysis of [γ-$^{32}$P]ATP or [α-$^{32}$P]dATP by the recA(K72R) protein was monitored by the time-dependent production of $^{32}P_i$ or [α-$^{32}$P]dADP, respectively, using polyethyleneimine-cellulose thin-layer chromatography and was quantitated using a Betagen Betascope 603 radioisotopic analyzer. Unless noted, assays were conducted at 37 °C in 25 mM Tris acetate (pH 7.5), 6 mM magnesium acetate, and 0.1 mM dithiothreitol using 1 mM ATP or dATP.

*DNA Strand Exchange Assay*—The agarose gel assay for DNA strand exchange was conducted, visualized, and quantitated as described previously (36). Reactions contained a regenerating system consisting of 20 units of pyruvate kinase/ml. The extent of DNA heteroduplex formation was determined by quantifying the S1 nuclease (Pharmacia) sensitivity of the displaced linear ssDNA by using tritiated linear M13 dsDNA substrate (1). *Sss*I methylase (New England Biolabs, Inc.) in the presence of *S*-[*methyl*-$^3$H]adenosyl-L-methionine (Du Pont-New England Nuclear) was employed to $^3$H-methylate the linear M13 dsDNA.

## RESULTS

*NTP Hydrolysis by recA(K72R) Protein Is Dramatically Attenuated Relative to That of Wild-type recA Protein*—To assess the effect of the conservative arginine mutation within the putative A site of the recA protein, ATP and dATP hydrolyses by the recA(K72R) protein were measured. As depicted in Fig. 1*A*, the recA(K72R) protein hydrolyzes both ATP and dATP, displaying a sigmoid dependence on NTP concentration that is characteristic of the recA protein. However, the $k_{cat}$ for both ATP (0.032 min$^{-1}$) and dATP (0.052 min$^{-1}$) hydrolyses is reduced 850- and 600-fold, respectively, when compared to that of the wild-type recA protein. This reduced level of NTP hydrolysis by the recA(K72R) protein is also manifest (data not shown) under numerous conditions known to stimulate wild-type recA protein activity (*i.e.* various concentrations of DNA (M13 ssDNA, poly(dT), and M13 dsDNA), a range of magnesium ion concentrations (2–10 mM), a range of ATP and dATP concentrations (1–5 mM), high salt activation (1.8 M sodium acetate or sodium chloride) (34), a range of pH (6.2–8.0), and under DNA strand exchange conditions), confirming that the mutation of lysine 72 to arginine dramatically attenuates NTP hydrolysis.

The ssDNA-dependent hydrolysis of dATP by the recA protein is indicative of ternary complex formation consisting of dATP, the recA protein, and ssDNA. The *E. coli* SSB protein facilitates formation of this ternary complex and thereby stimulates the M13 ssDNA-dependent dATP hydrolysis activity of the recA protein by allowing access to regions of ssDNA involved in secondary structure that limit the binding of the recA protein (35). To ensure that the NTP hydrolysis observed is intrinsic to the mutant recA(K72R) protein, the stoichiometry of ternary complex formation was measured. As illustrated in Fig. 1*B*, the M13 ssDNA-dependent rate of dATP hydrolysis increases linearly with

[2] W. M. Rehrauer and S. C. Kowalczykowski, unpublished results.
[3] S. C. Kowalczykowski, unpublished protocol.

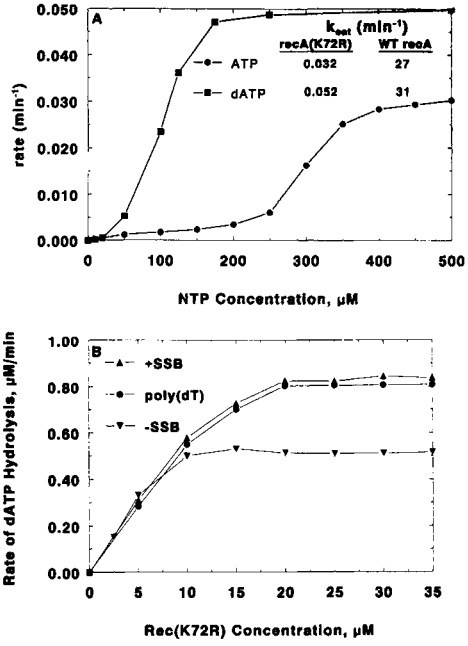


FIG. 1. *A*, dependence of recA(K72R) protein NTP hydrolysis activity on ATP and dATP concentrations. The rate of ATP or dATP hydrolysis by the recA(K72R) protein is given as moles of NTP hydrolyzed per minute/mole of protein. The concentration of the recA(K72R) protein in the assays was 20 μM, whereas the concentration of poly(dT) was 140 μM. For comparison, the turnover numbers ($k_{cat}$) for the wild-type (*WT*) recA protein are 27 and 31 $min^{-1}$ for ATP and dATP, respectively. *B*, effect of the *E. coli* SSB protein on the M13 ssDNA-dependent hydrolysis of dATP as a function of the recA(K72R) protein concentration. The concentration of M13 ssDNA and poly(dT) was 60 μM. SSB protein, when present, was added at a concentration of 3.3 μM to the reaction mixture last. ▼ and ▲, reactions using M13 ssDNA in the absence and presence of SSB protein, respectively; ●, reactions employing poly(dT) as the ssDNA substrate in the absence of SSB protein.

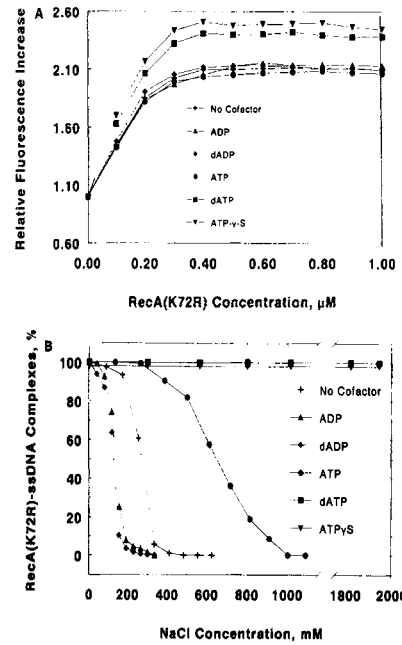


FIG. 2. *A*, binding of the recA(K72R) protein to εM13 DNA in the absence and presence of various nucleotide cofactors. recA(K72R) protein titrations were carried out by adding aliquots of recA(K72R) protein stock to 3 μM εM13 DNA in the absence or presence of various nucleotide cofactors. The relative fluorescence increase is defined as the ratio of the fluorescence of the recA protein-εM13 DNA complex relative to the protein-free DNA (6). *B*, effect of nucleotide cofactors on the stability of recA(K72R) protein-εM13 DNA complexes to dissociation by NaCl. Salt titrations were conducted by adding concentrated NaCl to recA(K72R) protein-εM13 DNA complexes, which consisted of 1 μM recA(K72R) protein and 3 μM εM13 DNA, in the absence or presence of various nucleotide cofactors.

recA(K72R) protein concentration until a plateau is achieved (~0.5 μM dATP/min), corresponding to an apparent stoichiometry of 7 nucleotides/recA(K72R) protein monomer. Addition of SSB protein to the M13 ssDNA-dependent reaction or substitution of poly(dT), which is devoid of secondary structure, for the M13 ssDNA increased both the observed rate of hydrolysis (~0.9 μM dATP/min) and the amount of the recA protein needed for saturation of the DNA (≈4 nucleotides/recA(K72R) protein momomer) by ~2-fold. This behavior is characteristic of the recA protein and clearly demonstrates that the observed NTP hydrolysis activity, although greatly diminished relative to that of the wild-type recA protein, is intrinsic to the recA(K72R) protein.

*recA(K72R) Protein Responds Allosterically to Nucleotide Cofactors*—The structure of recA protein-ssDNA complexes is allosterically moderated through the binding of nucleotide cofactor (3–5). Fig. 2*A* depicts the binding of the mutant recA(K72R) protein to ssDNA and its response to various nucleotide cofactors using εM13, a modified M13 ssDNA whose fluorescence is enhanced upon recA protein binding (37). In the absence or presence of various nucleotide cofactors, the recA(K72R) protein binds εM13 DNA with virtually the same stoichiometry (8 nucleotides/protein monomer) as the wild-type recA protein (data for wild type not shown). The relative fluorescence increase (RFI) for the recA(K72R) protein-εM13 DNA complex formed in the presence of dATP and ATPγS cofactors is high (2.4), corresponding to an extended functional nucleoprotein filament, one of the hallmarks of the high affinity DNA binding state of the recA protein (6). Complex formation with the mutant protein in the absence of cofactor or in the presence of either ADP or dADP results in a low RFI value (2.0), indicative of a compact nonfunctional nucleoprotein filament (3, 6). These results demonstrate that the recA(K72R) protein not only binds ssDNA, but also responds allosterically to nucleotide cofactor binding. However, in contrast to the wild-type recA protein, ATP does not induce the high RFI (*i.e.* extended) conformer of the recA(K72R) protein.

*ssDNA Binding Affinity of recA(K72R) Protein Is Modulated by Nucleotide Cofactors*—In addition to the structural transitions induced by nucleotide cofactors, the binding affinity of the recA protein for ssDNA is also moderated by nucleotide cofactors. ATP increases and ADP decreases the affinity of the recA protein for ssDNA (3); these changes are manifest in the NaCl concentration required to dissociate one-half of the recA protein-εM13 DNA complex (referred to as the salt titration midpoint (STMP)) (3). As shown in Fig. 2*B*, the STMP for the recA(K72R) protein in the absence of cofactor is 280 mM NaCl. Both ADP and dADP decrease the affinity of the recA(K72R) protein for ssDNA (STMP = 150 mM NaCl), whereas ATP (STMP = 700 mM NaCl), dATP, and ATPγS (both STMPs > 1.9 M NaCl) increase the affinity of the recA(K72R) protein for ssDNA. Therefore, the ssDNA binding affinity of the recA(K72R) protein is modulated by nucleotide cofactors in a manner paralleling that of the wild-type recA protein.

*Binding Affinity for NTP of recA(K72R) Protein Is Similar to That of Wild-type recA Protein*—Allosteric induction of both the high RFI conformation and the increased ssDNA binding affinity in the presence of dATP and ATPγS indicates that the recA(K72R) protein binds dATP and ATPγS.

Despite the observation that the high RFI conformer of the recA(K72R) protein is not induced in the presence of ATP, the recA(K72R) protein must bind ATP since its affinity for ssDNA is increased by ATP and ATP is hydrolyzed. To quantify NTP binding, the fluorescent enhancement upon NTP binding to the recA protein-$\epsilon$M13 complexes was measured. As depicted in Fig. 3, the fluorescence (*i.e.* complex formation) increases in a sigmoidal manner with NTP concentration. The dATP or ATP$\gamma$S concentration required to achieve the half-maximal fluorescence enhancement is slightly greater (~2- and 4-fold, respectively) for the recA(K72R) protein than for the wild-type recA protein. Induction of the high RFI conformer of the recA(K72R) protein by ATP is not detected up to 5 mM ATP (data >150 $\mu$M not shown).

*recA(K72R) Protein Promotes Homologous Pairing and Stable Joint Molecule Formation in Presence of dATP but Not ATP*—Although deficient in NTP hydrolysis, the recA(K72R) protein is nearly wild type with respect to ssDNA and NTP cofactor binding and to allosterism induced by nucleotide cofactor binding. These properties permit evaluation of the role of NTP hydrolysis in the homologous pairing and exchange of DNA strands. Fig. 4 illustrates the ability of the recA proteins to promote DNA strand exchange. As expected, joint molecule formation by the recA(K72R) protein is not observed in the presence of ATP nucleotide cofactor (Fig. 4, *panel I, right*). However, in the presence of dATP (Fig. 4, *panel II, right*) and in the presence of ATP$\gamma$S,[2] the protein can promote stable (plectonemic) joint molecule formation. Fig. 4 shows that joint molecule formation in the presence of dATP is efficient, involving 80–90% of the input dsDNA, and occurs at a rate that is ~2-fold slower than that of the wild-type protein. In distinct contrast to the reaction promoted by the wild-type protein, the joint molecules are not converted to the complete strand-exchanged heteroduplex DNA product (*i.e.* gapped circular dsDNA); but, instead, they accumulate. This result is similar to that obtained with the wild-type recA protein in the presence of ATP$\gamma$S, suggesting that ATP hydrolysis is important for DNA heteroduplex extension (1).

The extent of DNA heteroduplex formation was quantified using the S1 nuclease assay. The average size of the DNA heteroduplex joint formed by the recA(K72R) protein in the presence of dATP is ~1500 base pairs and, within experimental error, is invariant with time (Fig. 5). This suggests that the homologous encounter results in the initial formation of a joint molecule containing ~1500 base pairs of heteroduplex DNA that is not subsequently extended by the recA(K72R) protein.

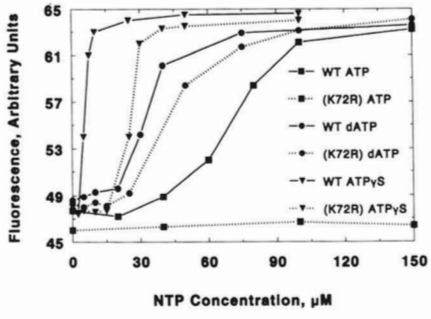


FIG. 3. **Binding of nucleotide cofactors by recA(K72R) protein.** Aliquots of concentrated ATP, dATP, or ATP$\gamma$S stock were added to wild-type (*WT*) recA protein- or recA(K72R) protein-$\epsilon$M13 complexes preformed using 1 $\mu$M recA protein and 3 $\mu$M $\epsilon$M13, and the fluorescence increase was measured.

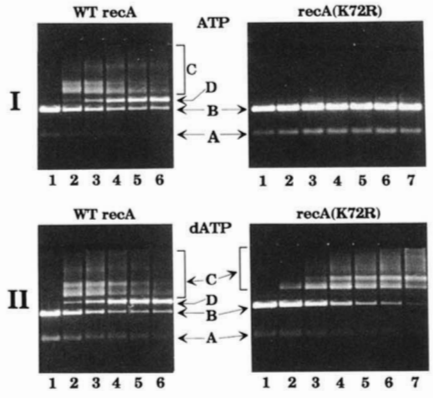


FIG. 4. **Wild-type recA protein- and recA(K72R) protein-promoted DNA strand exchange.** Shown is the time course for DNA strand exchange promoted by the wild-type (*WT*) recA protein (*left panels*) or the recA(K72R) protein (*right panels*) in the presence of ATP (*panel I*) or dATP (*panel II*) cofactors. *Lanes 1–7* denote 0, 10, 20, 40, 60, 90, and 180 min, respectively. The substrates of the reaction are circular M13 ssDNA (*A*) and M13 dsDNA linearized with *Eco*RI restriction endonuclease (*B*). The joint molecule intermediate species (*C*) contain both strands of a linear M13 dsDNA molecule and the invading circular M13 ssDNA molecule. Indicative of the complete exchange of DNA strands, the product is a gapped circular heteroduplex DNA molecule (*D*).

## DISCUSSION

Acknowledging that "function" follows "form," we sought to define the NTP hydrolytic domain of the recA protein. We hoped to address further both the issue of energy transduction by the recA protein and the significance of the allosteric conversion between active (ATP-bound) and inactive (ADP-bound) recA protein conformations in the mechanism of pairing and exchange of DNA strands. By engineering a conservative mutation (lysine 72 to arginine) within a highly conserved region of the recA protein (2) that bears primary sequence similarity to that of the A site motif of Walker *et al.* (20), we discovered that the NTP hydrolysis activity of the resultant recA(K72R) protein is attenuated minimally 600-fold relative to that of the wild-type recA protein. This dramatic reduction in NTP hydrolysis identifies lysine 72 as a component of the catalytic domain. This identification is supported conclusively by the concurrent determination of the crystal structure of the recA protein in the presence of ADP; amino acid residues comprising the A site of the recA protein ($^{66}$GPESSGKT$^{73}$) interact with the phosphate chain

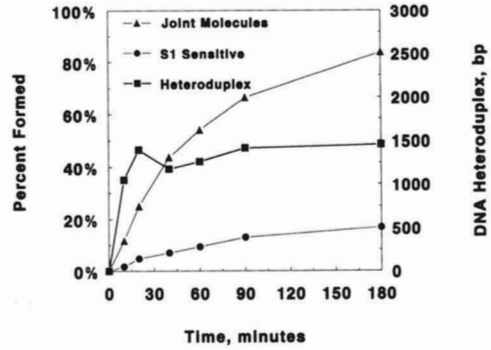


FIG. 5. **DNA heteroduplex formation by recA(K72R) protein.** DNA strand exchange reactions in the presence of dATP were conducted as described, except that the linear M13 dsDNA substrate was tritium-labeled. Time points were equally divided for analysis by either gel electrophoresis (percent joint molecules formed) or S1 nuclease susceptibility (percent S1 nuclease-sensitive). The S1 nuclease assays and the resultant determination of the extent of DNA heteroduplex (base pairs) were done as previously described (1).

of a bound ADP molecule; and the structure of this region is nearly identical to the A sites of the adenylate kinase, elongation factor Tu, and p21 ras proteins (18, 19).

Despite the dramatic attenuation in NTP hydrolysis activity, the ssDNA binding affinity of the recA(K72R) protein and its modulation by nucleotide cofactors are virtually identical to those of the wild-type recA protein. ATP, dATP, and ATPγS increase the affinity of the recA(K72R) protein for ssDNA, whereas ADP and dADP decrease the ssDNA binding affinity of the recA(K72R) protein (Fig. 2*B*). The structure of recA(K72R) protein-εM13 DNA complexes is also affected by the binding of nucleotide cofactors dATP and ATPγS, as evidenced by the enhancement of the relative fluorescence increase (RFI = 2.4) (Fig. 2*A*). This fluorescence increase directly parallels the formation of an extended functional nucleoprotein filament (6). However, in contrast to the wild-type recA protein, the recA(K72R) protein responds asymmetrically to ATP in that even though ATP increases its affinity for ssDNA, ATP does not induce the high RFI extended conformer. This differential allosteric effect of ATP relative to dATP and ATPγS on the recA(K72R) protein is unexpected as neither the 2′- nor 3′-OH of ADP appears to interact with the recA protein (38). This result, however, suggests that different contacts are made with the nucleoside in the high affinity state of the recA protein and that these changes involve the phosphate-binding loop; in agreement, the structural changes in the ras p21 protein (16) that accompany GTP hydrolysis are mediated via changes in hydrogen bonding between the γ-phosphate and amino acid residues in the nucleotide-binding domain. With the single exception of the failure to form the extended (high RFI) conformer in the presence of ATP, the recA(K72R) protein responds allosterically to various nucleotide cofactors in a fashion paralleling that of the wild-type recA protein.

Characteristic of the wild-type recA protein (39, 40), both NTP hydrolysis and NTP cofactor binding of the recA(K72R) protein display cooperative behavior. The nucleotide concentration dependence of ATP and dATP hydrolyses yields apparent $S_{0.5}$ values of 300 and 100 μM, respectively, which are virtually identical to (for dATP) or within 2–3-fold (for ATP) of the values previously reported for wild-type recA protein (39, 40). Additionally, the affinities of the recA(K72R) protein-ssDNA complex for ATPγS and dATP, examined by induction of the high RFI conformer, are within 2–4-fold of corresponding wild-type values. These data argue that the capacity and properties of nucleotide cofactor binding are minimally perturbed by the lysine to arginine mutation.

An *in vitro* reaction that represents a step of genetic recombination *in vivo* is the recA protein-promoted homologous pairing and subsequent exchange of a circular ssDNA molecule for its homologue within a linear dsDNA molecule (41, 42). Upon complete exchange of DNA strands, the final products of this reaction are nicked circular heteroduplex DNA and linear ssDNA. The recA(K72R) protein is unable to promote joint molecule formation in the presence of ATP because, although ATP increases the ssDNA binding affinity, it does not induce the extended functional conformation (high RFI) of the recA(K72R) protein. This result substantiates the need for both components of the high affinity binding state of the recA protein in the homologous pairing of DNA strands (6). In contrast, in the presence of dATP, the recA(K72R) protein promotes joint molecule formation with up to 1.5 kilobase pairs of DNA strands being exchanged; however, complete exchange to form the gapped circular heteroduplex DNA product is not exhibited. This observation is in complete agreement with and extends previous results (1, 7) obtained using the nonhydrolyzable ATP analogue ATPγS, indicating that recA protein-mediated NTP hydrolysis is not obligatorily linked to joint molecule formation or to the exchange of DNA strands. As previously proposed, NTP hydrolysis may simply function to generate the low affinity ADP- or dADP-bound form of the recA protein, which facilitates dissociation and recycling of the recA protein. This cyclic process, coupled to NTP hydrolysis, permits the subsequent complete exchange of DNA strands; without NTP hydrolysis, only one cycle of DNA strand exchange is possible. Thus, although ATP hydrolysis is not essential to the actual mechanics of DNA strand exchange, ATP hydrolysis plays an important role in DNA heteroduplex extension (6). Since complexes of either the wild-type recA protein and ATPγS or the recA(K72R) protein and dATP are compromised in their ability to dissociate and recycle due to either the nature of the cofactor or the hydrolysis deficiency of the protein, respectively, they are unable to process further the joint molecules formed. Biochemical characterization of the recA(K72R) protein in the DNA strand exchange reaction confirms the essential role of the allosteric transition to the ssDNA high affinity binding state of the recA protein in both homologous pairing and stable joint molecule formation and affirms the absence of a direct mechanistic requirement for recA protein-mediated NTP hydrolysis in the homologous pairing of DNA strands. Thus, NTP hydrolysis need not be a universal hallmark of homologous pairing and DNA strand exchange proteins (8–12).

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