Relationship of the Physical and Enzymatic Properties of *Escherichia coli* RecA Protein to Its Strand Exchange Activity†

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Received March 21, 1986; Revised Manuscript Received July 21, 1986

ABSTRACT: We have shown that performing the recA protein catalyzed strand exchange reaction in the presence of acetate anions, rather than chloride which is commonly used, greatly increases the rate of the reaction. The initial rate of the reaction in an acetate-based buffer is approximately 3–4 times higher in the presence of *Escherichia coli* single-stranded DNA binding protein (SSB protein) and 2 times higher in its absence than the initial rate in chloride. To determine the enzymatic basis for this stimulatory effect of acetate buffer, we investigated the relationship between a number of physical and enzymatic properties of recA protein and the strand exchange reaction. We have found that although the acetate anion has some effect on the aggregation properties and the single-stranded DNA-dependent ATPase activity of recA protein, these effects cannot explain the enhanced strand exchange activity in an acetate-based buffer. We do find, however, that two aspects of recA protein activity closely parallel the ability of this protein to catalyze strand exchange. The first is the ability of recA protein to displace SSB protein from single-stranded DNA, an event critical to presynaptic complex formation. RecA protein is able to resist displacement by SSB protein at a lower magnesium concentration in acetate than in chloride buffer. The magnesium ion concentration dependence of strand exchange coincides exactly with this behavior. The second activity correlated to strand exchange is the duplex DNA-dependent ATPase activity of recA protein. We find that over a wide variety of sodium chloride and sodium acetate concentrations, this duplex DNA-dependent ATPase activity is linearly related to the amount of product formed in the strand exchange reaction. We postulate that this duplex DNA-dependent ATPase activity is important in the denaturation of the duplex DNA during the branch migration step of strand exchange and have also determined that this reaction is quite efficient, with the number of ATP molecules hydrolyzed per base pair exchanged being 0.75 ± 0.25. In addition, recA protein catalyzed strand exchange between circular single-strand and linear duplex DNA molecules is shown to be irreversible, and a possible explanation for this irreversibility is presented.

RecA protein is a 37.8-kilodalton (kDa)[1] protein that has been shown to play a vital role in genetic recombination [for reviews, see McEntee & Weinstock (1981), Radding (1982), and Dressler & Potter (1982)]. RecA mutants are phenotypically deficient in recombination by as much as 6 orders of magnitude and show an increased sensitivity to UV irradiation. This sensitivity is due partly to the role that recA protein plays in SOS induction where it catalyzes the proteolytic cleavage of lexA protein in the presence of ATP and single-stranded DNA [for a review, see Little & Mount (1982)].

In vitro, recA protein catalyzes the assimilation of DNA with single-stranded character into a homologous duplex in the presence of either SSB protein (Cox & Lehman, 1981) or bacteriophage T4 gene 32 protein (Weinstock et al., 1982). In the absence of either SSB or T4 gene 32 proteins, the rate and yield of the reaction are approximately 2–3-fold lower (Cox & Lehman, 1981; Kahn & Radding, 1984). The strand exchange reaction is dependent on ATP hydrolysis (West et al., 1981b; Cox & Lehman, 1981) and requires that one of the DNA molecules used as a substrate has single-stranded character at a region of homology (Cassuto et al., 1980; Das Gupta et al., 1981). Also, due to topological constraints, complete strand exchange cannot occur unless one of the duplex molecules has an end (Cunningham et al., 1980). DNA molecules commonly used as substrates in the exchange reaction are linear duplex DNA and homologous circular single-stranded DNA (Cox & Lehman, 1981). The resulting product is a nicked circle which is easily distinguishable from either of the initial substrates on an agarose gel.

The D-loop formation and strand exchange reactions are inhibited by 50 mM sodium chloride or by the presence of ADP (Cox & Lehman, 1982; Shibata et al., 1981). Similarly, DNA binding studies have shown that recA protein binds to single-stranded DNA with a lower affinity in the presence of ADP or at increased sodium chloride concentrations (Menetski & Kowalczykowski, 1985). Thus, there may be some relationship between the stability of the single-stranded DNA–recA protein complex and the ability of recA protein to catalyze the strand exchange reaction. Since the conditions that promote weak DNA binding are similar to the conditions that inhibit catalytic activity, it is possible that conditions favoring tighter DNA binding may also be conducive to an increase in the rate of the strand exchange reaction. The DNA binding studies have also shown that recA protein binds to single-stranded DNA approximately 10 000-fold more tightly in a

† This work was supported in part by funds from National Institutes of Health Grant AI-18987 and from the Earl M. Bane Biomedical Research Fund and by an American Cancer Society Junior Faculty Fellowship (JFRA-70).

[1] Abbreviations: SSB protein, *E. coli* single-stranded DNA binding protein; RF, replicative form; DTT, dithiothreitol; SDS, sodium dodecyl sulfate; ATP, adenosine 5′-triphosphate; EDTA, ethylenediaminetetraacetate; NADH, nicotinamide adenine dinucleotide (reduced); kDa, kilodalton(s); ODU, optical density unit(s); Tris, tris(hydroxymethyl)-aminomethane.

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buffer containing acetate ions than it does in one containing chloride ions (Menetski & Kowalczykowski, 1985). Thus, if single-stranded DNA binding affinity is related to the efficiency of strand exchange, then a recA protein reaction buffer containing acetate as the anion, rather than the more commonly used chloride, may improve the rate of the reaction both in the presence and in the absence of additional proteins (e.g., SSB protein).

In this paper, we describe an acetate-based buffer system in which the rate of product formation in the strand exchange reaction involving linear duplex and circular single-stranded DNA molecules, in the presence of SSB protein, is shown to be approximately 3–4 times that in chloride buffer. We have also attempted to determine which physical or enzymatic property of recA protein is rate limiting for product formation in the strand exchange reaction by quantitatively comparing the extent of product formation with various properties of recA protein (i.e., single-stranded DNA binding, aggregation, single-stranded DNA-dependent ATPase activity, and double-stranded DNA-dependent ATPase activity). We find that the effectiveness of recA protein in catalyzing this reaction is dependent to some degree on its ability to resist displacement by SSB protein and correlates well with its double-stranded DNA-dependent ATPase activity. Our data suggest that the duplex DNA-dependent ATPase activity of recA protein is responsible for the DNA unwinding that occurs during branch migration. It is also demonstrated that, contrary to expectations, the reaction is irreversible rather than at equilibrium with these particular substrates. A possible cause for the irreversibility of this reaction is discussed.

Materials and Methods

Protein and DNA Isolation. Both single- and double-stranded DNA molecules were isolated from bacteriophage M13 mp7 by using the procedures of Messing (1983). The molar concentrations were determined by using extinction coefficients of 6500 M^{-1} cm^{-1} for duplex DNA and 8780 M^{-1} cm^{-1} for single-stranded DNA at 260 nm. The duplex DNA was linearized by digesting with *Eco*RI to remove the 42 base pair mp7 cloning insert.

RecA protein was isolated according to the procedure of Cox et al. (1981). SSB protein was purified from strain RLM727 by using a preparative protocol provided by Dr. Roger McMackin of The Johns Hopkins University. We are grateful to Dr. McMackin for providing both the strain and the purification procedure. The protein concentrations were determined by using molar extinction coefficients of 2.7×10^4 M^{-1} cm^{-1} for recA protein and 3×10^4 M^{-1} cm^{-1} for SSB protein, both at 280 nm (Ruyechan & Wetmur, 1976).

Reaction Conditions. The reaction mixture for the strand exchange assays consisted of 25 mM Tris–acetate or Tris-HCl (pH 7.5), 10 mM magnesium acetate or magnesium chloride, 3.7% glycerol, 1 mM DTT, 1 mM ATP, 9.9 μM M13 single-stranded DNA, 16.8 μM M13 linear duplex DNA, 6 μM recA protein, 0.9 μM SSB protein, and an ATP regenerating system consisting of 5–10 mM phosphoenolpyruvate and 2 units/mL pyruvate kinase, unless indicated otherwise. The reaction was initiated by the simultaneous addition of recA and SSB proteins after preincubation of all other components for 2 min at 37 °C. When salt or magnesium ion concentrations were varied, the mono- or divalent cations were added directly to the buffer before the addition of any other components. All reactions took place at 37 °C.

Agarose Gel Assay. The formation of product molecules was measured by using the agarose gel electrophoresis assay described by Cox and Lehman (1981). Samples (40 μL) of the above reaction mix were stopped by the addition of 1.2 μL of 0.5 M EDTA and 4.6 μL of 10% SDS at indicated time points. These samples were loaded onto a 0.8% agarose gel and electrophoresed in TAE buffer (0.04 M Tris–acetate and 0.002 M EDTA). Due to the fact that linearization of the replicative form of M13 mp7 results in the removal of a 42 base pair insert, one of the product molecules in our experiments is a gapped duplex rather than the nicked duplex in the original Cox and Lehman (1981) studies.

The extent of product formation was quantified by scanning a photographic negative of the gel with a Zeineh soft laser scanning densitometer (Biomed Instruments, Inc.) connected to a Hewlett Packard 3390A integrator. The percent product was defined as the amount of gapped circular DNA present divided by the sum of the amounts of gapped circular and linear duplex DNA present in a given lane.

D-Loop Assay. D-loop formation was monitored with the nitrocellulose filter assay described by Cox and Lehman (1981). The reaction, typically containing 1500 cpm of [^{3}H]thymine-labeled duplex DNA in the above reaction mix, was stopped by adding aliquots (50 μL) to 2.5 μL of 10% SDS and 5 μL of 0.125 M EDTA at the indicated time points. After 20 s, 1 mL of D-loop buffer (2 M sodium chloride and 0.15 M sodium citrate) was added and the entire mixture put on ice. This mixture was then filtered within 10 min on a 0.45-μm nitrocellulose filter (Schleicher & Schuell, grade BA85, 24-mm diameter) and washed with 2 mL of D-loop buffer. The filters were dried and counted for 1 min in 4a20 scintillation cocktail (RPI) in a Beckman LS 7800 scintillation counter.

Aggregation Assay. The aggregation of recA protein was determined by measuring the light scattering caused by the increase in turbidity that accompanies aggregation (Cotterill & Fersht, 1983). RecA protein (6 μM) was added to a cuvette containing 25 mM Tris–chloride or –acetate, 3.7% glycerol, and 1 mM DTT and preincubated at 25 or 37 °C, as indicated. Increments of 1 mM magnesium chloride or magnesium acetate were added by a Hamilton Microlab P microprocessor-controlled micropipet, and the increase in light scattering was measured by using a Perkin-Elmer MPE-44E fluorescence spectrophotometer (excitation and emission wavelengths set to 350 nm) interfaced to a Hewlett Packard 85 microcomputer. Salts (sodium chloride and sodium acetate) were added and the results measured in the same manner. Experiments were performed in both the presence and absence of ATP (with a regenerating system) and DNA, as indicated.

ATPase Assay. ATPase activity was measured according to the procedure described by Kreuzer and Jongeneel (1983) and adapted for use with recA protein by Kowalczykowski and Krupp (1986). Hydrolysis of ATP to ADP and P_i is linked to the oxidation of NADH to NAD and monitored by a decrease in the absorbance at 340 nm as measured by a Hewlett Packard 8450 spectrophotometer. The raw data shown (i.e., Figure 8) represent the depletion of NADH, which is directly proportional to the amount of ATP hydrolyzed. The final plateau reached represents the exhaustion of NADH. A decrease of 1 ODU is equivalent to the hydrolysis of 0.16 mM ATP. The rate of ATP hydrolysis was determined from the linear region of the data by using the dA/dT software key provided on the HP8450. The assays were performed at 37 °C using 6 μM recA protein and either 9.9 μM M13 single-stranded DNA or 16.8 μM *Eco*RI-cut M13 RF DNA in the single-stranded DNA-dependent or duplex DNA-dependent ATPase assays, respectively. SSB protein was added, where indicated, to a final concentration of 0.9 μM. The assay buffer

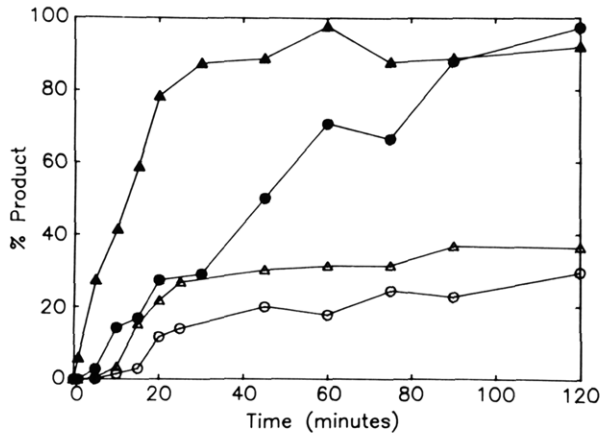

FIGURE 1: Comparison of strand exchange in chloride and acetate buffers. Chloride, no SSB protein (○); acetate, no SSB protein (△); chloride + SSB protein (●); acetate + SSB protein (▲). Standard strand exchange reaction conditions were used. RecA protein and SSB protein were added simultaneously after preincubation of all other components.

contained 25 mM Tris-HCl or Tris–acetate (pH 7.5), 10 mM magnesium chloride or magnesium acetate, 1.0 mM DTT, 5 mM phosphoenolpyruvate, 1.0 mM ATP, 100 μg/mL NADH, and approximately 20 units/mL each of lactate dehydrogenase and pyruvate kinase.

RESULTS

Acetate Buffer Increases the Rate of Product Formation in the RecA-Catalyzed Strand Exchange Reaction 3–4-Fold. To determine whether the substitution of acetate anions for chloride anions had an effect on the strand exchange activity of recA protein, agarose gel assays were performed. This activity was measured in an acetate buffer (containing Tris–acetate and magnesium acetate) and compared to that obtained in a chloride buffer (containing Tris–chloride and magnesium chloride). To quantitate these results, a photographic negative of the gel was scanned, and the results are shown in Figure 1. In the absence of SSB protein, the reaction rate, as measured by the initial appearance of the gapped circular DNA product, is approximately 2-fold higher in acetate than in chloride buffer under conditions which are otherwise identical. The product yield is only 30–40% after 2 h, and reaction times of up to 4 h fail to produce more than 30–40% product in either chloride or acetate buffers.

The effect of SSB protein on this reaction was examined as well, and these results are also shown in Figure 1. In chloride buffer, the addition of SSB protein increases the rate and yield of the gapped circular DNA product approximately 2–3-fold relative to those obtained in the absence of SSB protein, an effect that has been previously reported by Cox and Lehman (1981). The initial rate of the reaction in chloride buffer, as measured by the concentration of product formed up to 60 min, is 0.2 μM gapped duplex DNA formed per minute (or approximately three nucleotides incorporated per second). In acetate buffer, the yield in the presence of SSB protein is also increased approximately 2–3-fold over that observed in the absence of SSB. The initial rate achieved in this system, averaged over the first 20 min of reaction time, is 0.66 μM gapped duplex DNA formed per minute (or approximately nine nucleotides incorporated per second), an increase of 3.3-fold relative to the rate in chloride buffer.

Strand Exchange Involving Linear Duplex and Circular Single-Stranded DNA Is Irreversible. The observation that the product yield in the strand exchange reaction approaches 100% in the presence of SSB is unexpected. If it is assumed

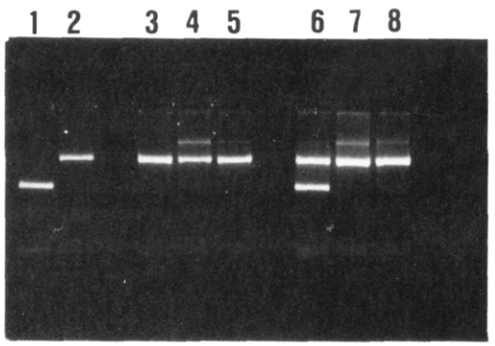

FIGURE 2: Irreversibility of recA strand exchange reaction using linear duplex and circular single strand as substrates. Lanes 1 and 2 are 0- and 90-min time points of the reaction involving the initial substrates, linear duplex and circular single-stranded DNA molecules. Lanes 3, 4, and 5 are 0-, 30-, and 60-min time points of the reaction involving gapped circle and linear single strand as substrates in the presence of the standard amount of recA and SSB proteins. Lanes 6, 7, and 8 are 0-, 30-, and 60-min time points after the readdition of linear duplex and circular single strand along with 2× the standard amount of recA and SSB proteins. Standard strand exchange reaction conditions were used. RecA protein and SSB protein were added simultaneously after preincubation of all other components.

that the products could also participate in strand exchange to re-form the initial substrates, an equilibrium situation with a yield of approximately 50% product should occur. To ensure that the lack of a 50% equilibrium between product and substrate molecules was not the result of an intrinsic difference in their stabilities (even though the number of base pairs formed in each is identical), renaturation studies of the substrate molecules were performed. When a mixture of the linear duplex and single-stranded DNA molecules (under conditions identical with those in Figure 1 but in the absence of any proteins) was heat denatured at 95 °C and allowed to slowly renature, first at the melting temperature (85 °C) and then at 65 °C, a 50:50 mixture of linear duplex and gapped duplex molecules was formed (not shown). Thus, the lack of a 50:50 equilibrium in the presence of protein is not a property intrinsic to the DNA molecules and must, therefore, reflect some aspect of the protein-catalyzed reaction.

The unexpectedly high yield of this reaction might also be due to an irreversible (on this time scale) binding of proteins to the product molecules, somehow rendering them inactive with regard to strand exchange in the reverse direction. Soltis and Lehman (1983) have shown that after the strand exchange reaction has occurred, recA protein will cosediment with the nicked circular product molecules, which might result in an inactivation of the product molecules. Such an inactivation may also correspond to the phenomenon of duplex DNA "loading" which has been reported by Shibata et al. (1982). If protein inactivation were responsible, then using protein-free gapped circular and linear single-stranded DNA product molecules as substrates in a reaction should result in the formation of the initial substrates (linear duplex and circular single-stranded molecules).

To form these substrates, a standard strand exchange reaction was performed in acetate buffer in the presence of SSB to 100% completion (Figure 2, lanes 1 and 2). This was extracted with 1 volume of distilled phenol to remove all proteins, washed 3 times with 2 volumes of anhydrous ether, and ethanol precipitated to recover the DNA (gapped circular and linear single-stranded molecules). This DNA was resuspended in either chloride- or acetate-based reaction buffer and divided into two halves. In the first, recA and SSB proteins were added, and this mixture was incubated to allow the strand exchange reaction to occur. In either acetate

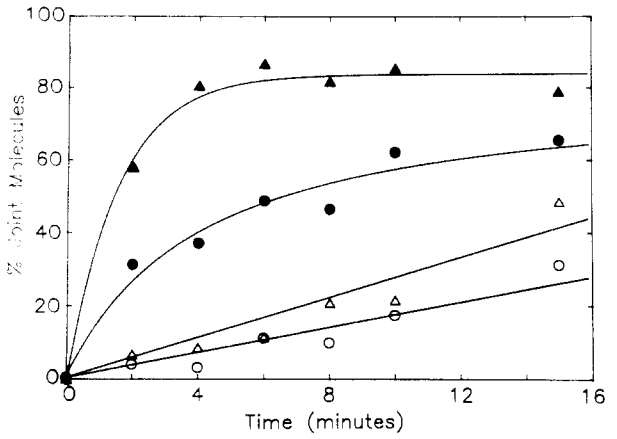

FIGURE 3: Comparison of D-loop formation in chloride and acetate buffers. Chloride, no SSB protein (○); acetate, no SSB protein (△); chloride + SSB protein (●); acetate + SSB protein (▲). Standard D-loop assay conditions were used. RecA protein and SSB protein were added simultaneously after preincubation of all other components.

(Figure 2, lanes 3–5) or chloride (not shown) buffer, no conversion of the gapped circular duplex to a linear duplex was observed. To ensure that some residue remaining from the phenol extraction was not responsible for this lack of reaction, the original substrates (i.e., linear duplex and circular single-stranded DNA) were introduced into the second half of the resuspended DNA along with 2× recA and SSB proteins to demonstrate that the "forward" reaction could still occur (Figure 2, lanes 6–8). The formation of additional gapped circular DNA product confirms that the reaction is able to occur in the phenol-treated mix. This phenomenon of irreversibility appears to be peculiar to the circular single-stranded and linear duplex substrates as attempts in this lab to duplicate this high product yield (i.e., 100%) using linear single-stranded and linear duplex DNA molecules as substrates have been unsuccessful.

Finally, contamination of the recA protein or SSB protein stocks by ligase activity, which might preferentially and irreversibly seal the ends of the circular duplex molecule (possibly brought together by the secondary structure of the mp7 palindromic sequence that is present in the circular DNA but absent in the linear molecule), was ruled out because removal of the terminal phosphates with calf intestinal phosphatase had no effect on the extent of the reaction (not shown).

Use of Acetate Buffers Also Affects the Formation of D Loops. To discover whether the initiation of recA protein catalyzed strand exchange is affected by the presence of acetate anions, D-loop assays were performed. D-loop formation represents an early step in the strand exchange reaction and is a measure of the number of duplex DNA molecules that have been infiltrated by a complementary single-stranded DNA molecule to form a heteroduplex molecule (Beattie et al., 1977; Das Gupta et al., 1981; Cox & Lehman, 1981). Figure 3 shows that the effect of acetate on the formation of D loops is similar to its effect on the formation of final product molecules as measured by the gel assay; that is, the initial rate of D-loop formation is higher in acetate than in chloride. This increase in rate of approximately 2-fold occurs both in the presence and in the absence of SSB protein. These results demonstrate that initiation is affected by the presence of acetate but does not rule out the possibility that branch migration is also anion sensitive.

Acetate Buffer Moderates the Effect of Salts on Strand Exchange and D-Loop Formation. Menetski and Kowalczykowski (1985) have demonstrated that recA protein will bind more tightly to a chemically modified etheno derivative of M13 single-stranded DNA in buffers containing acetate ions than in those with chloride ions. This effect was demonstrated by gradually raising the salt concentration of a solution of recA protein bound to single-stranded DNA and observing the point at which the complex dissociates. In acetate solutions, which promote tight binding, the recA protein–single-stranded DNA complex will not dissociate until relatively high salt concentrations are reached (approximately 840 mM sodium acetate), whereas, in the presence of chloride ions, the recA protein–DNA complexes are less stable and will dissociate at lower salt concentrations (approximately 280 mM sodium chloride). If the strand exchange and ATP hydrolysis activities are correlated to single-stranded DNA binding affinity, then it is expected that these activities will occur in acetate buffers at salt concentrations that would otherwise be inhibitory in chloride buffers.

Table I: Effect of Salt Concentration on Initial Rate of Strand Exchange[a]

anion	rel initial rate (%) at salt concn (mM) of 0	50	100	200
chloride	100	68	13	0
acetate	100	65	34	11

[a] The percentages shown represent the initial rate of product formation relative to the rate observed in the absence of salt. Product formation is defined as the amount of gapped duplex DNA product and was determined by the agarose gel assay. The absolute rates of product formation at 0 mM salt are 0.2 and 0.66 μM/min in chloride and acetate buffers, respectively.

As can be seen in Table I, strand exchange is inhibited to a lesser degree by increased sodium concentrations in acetate buffer than in chloride buffer. In both chloride and acetate buffers, the addition of 50 mM sodium salt is enough to partially inhibit the reactions to the same degree. However, in chloride buffer, 100 mM sodium chloride is sufficient for almost complete (i.e., 90%) inhibition. Inhibition of this extent is not observed in acetate buffer, however, until a salt concentration of 200 mM is reached. Strand exchange reactions carried out in mixed buffer systems (i.e., magnesium acetate and sodium chloride or magnesium chloride and sodium acetate) produce intermediate results such that the actual rate is always greater than that obtained with an all-chloride system but less than that obtained with an all-acetate system (not shown).

Shibata et al. (1981) have shown that the addition of 50 mM sodium chloride resulted in a 50% inhibition of heteroduplex formation while 100 mM sodium chloride resulted in almost complete inhibition. D-loop formation, however, occurs in acetate buffer at salt concentrations that are inhibitory to D-loop formation in chloride buffer (not shown). An increase of at least 2-fold in the sodium acetate concentration is needed to produce the amount of inhibition observed with sodium chloride. The addition of 100 mM sodium acetate resulted in only a 20% inhibition of D-loop formation, and 200 mM sodium acetate caused a decrease of about 90%. Thus, the trends observed for the effect of sodium acetate on the overall strand exchange reaction are also paralleled in D-loop formation.

Acetate Buffer Has Little Effect on Single-Stranded DNA-Dependent ATPase Activity. The relative single-stranded DNA-dependent ATPase activity of recA protein in increasing concentrations of sodium chloride or sodium acetate is shown in Figure 4. Increasing the sodium chloride concentration in the chloride-based buffer causes only a gradual

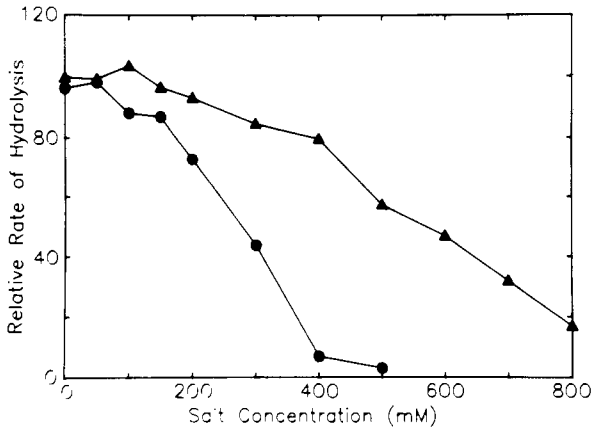

FIGURE 4: Effect of salt concentration on the rate of single-stranded DNA-dependent ATPase activity. Chloride (●); acetate (▲). Standard ATPase assay conditions were used. The absolute rates of ATP hydrolysis are 24.7 and 25.6 μM ATP/min at 0 mM salt for chloride and acetate buffers, respectively. The various salts were added directly to the buffer before the addition of any other components.

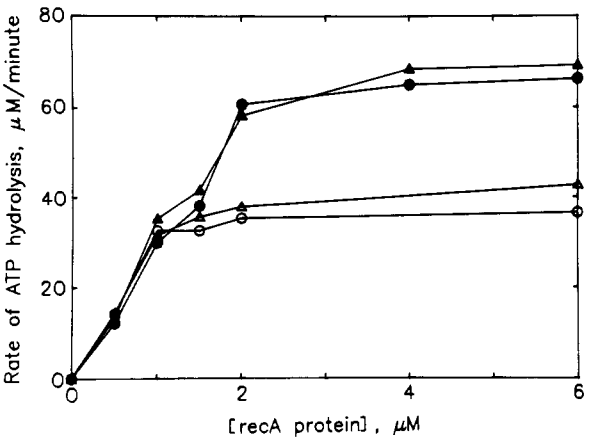

FIGURE 5: RecA protein concentration dependence of the single-stranded DNA-dependent ATPase activity. Chloride, no SSB protein (○); acetate, no SSB protein (△); chloride + SSB protein (●); acetate + SSB protein (▲). Standard ATPase assay conditions were used, with the exception of varying concentration of recA protein. For the data obtained in the presence of SSB protein, the reaction was allowed to proceed for 1 min in the absence of SSB protein. SSB protein was then added to a final concentration of 0.9 μM, and the steady-state reaction rate was measured.

decrease in hydrolysis until 200 mM sodium chloride where it then decreases rapidly. In comparison, an increase in the sodium acetate concentration to 200 mM results in little change, and significant inhibition does not begin until approximately 300–400 mM sodium acetate.

The results of these ATPase assays show that the inhibitory effect of increasing salt concentrations on the rate of strand exchange is not a consequence of the cessation of single-stranded DNA-dependent ATPase activity. Since strand exchange is inhibited by 90% in 100 mM sodium chloride or 200 mM sodium acetate, whereas ATPase activity is inhibited at much higher salt concentrations (350 mM sodium chloride or 800 mM sodium acetate), inhibition of strand exchange is not due to a limiting effect of salt concentration on the interaction of recA protein with single-stranded DNA. Instead, it must be due to an ionic interference of some other property of recA protein, such as an interaction with duplex DNA or with other recA protein molecules (e.g., aggregation; see below).

RecA Protein Exhibits the Same DNA Binding Stoichiometry in the Single-Stranded DNA-Dependent ATPase Assay Whether in Chloride or Acetate Buffer. The tighter single-stranded DNA binding in acetate may allow recA protein to more readily destabilize hairpins and other secondary structure in single-stranded DNA, provided that acetate does not equally affect the duplex DNA binding affinity. As a result, more single-stranded DNA would be readily available to recA protein, thus possibly increasing the rate and, in the case of strand exchange in the absence of SSB, the final product yield. This mechanism of destabilizing secondary structure was postulated by Muniyappa et al. (1983) for the action of SSB protein in the enhancement of strand exchange and has been confirmed by studies of the effects of SSB protein on the single-stranded ATPase activity of recA protein (Kowalczykowski & Krupp, 1986).

To probe the availability of single-stranded DNA to recA protein in chloride or acetate buffer, single-stranded DNA dependent ATPase activity was determined as a function of recA protein concentration. Since the ATPase activity is proportional to the concentration of the recA protein–single-stranded DNA complex it will reach a maximum value when recA protein has bound all available single-stranded DNA (Kowalczykowski & Krupp, 1986). If more single-stranded DNA is available to recA protein in acetate buffer, due to an increased ability to denature hairpins, this maximum hydrolysis rate will be increased, and the apparent stoichiometry at

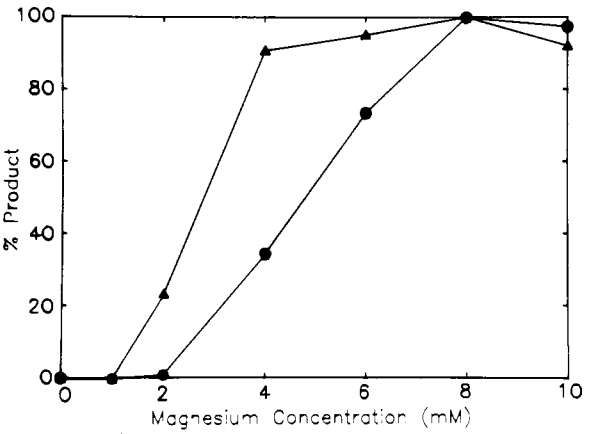

FIGURE 6: Effect of magnesium ion concentration on product formation in the strand exchange reaction. Chloride + SSB protein (●); acetate + SSB protein (▲). Standard strand exchange assay conditions were used, and the amount of product formed after 120 min is plotted. The various magnesium salts were added directly to the buffer before the addition of any other components. RecA protein and SSB protein were added simultaneously after preincubation of all other components.

saturation will decrease. As shown in Figure 5, both the maximum ATP hydrolysis rate and the apparent saturation stoichiometry both in the presence and in the absence of SSB protein are unchanged whether chloride buffer or acetate buffer is used. It is clear, however, that the addition of SSB protein both increases the maximum ATP hydrolysis rate in both buffers and alters the apparent site size of recA protein from 10 nucleotides/monomer, in the absence of SSB protein, to 5 nucleotides/monomer in its presence. These data indicate that there is little or no difference in either the availability of single-stranded DNA to recA protein or the ability of recA protein to destabilize secondary structure in chloride or acetate buffer.

Effect of Magnesium Ion Concentration on Strand Exchange and Presynaptic Complex Formation. The strand exchange reaction is dependent on the presence of magnesium, with optimal activity at 10 mM magnesium chloride in chloride buffer (Cox & Lehman, 1982). As Figure 6 shows, the optimal magnesium concentration in acetate buffer is broader, ranging from 4 to 10 mM, and more product is formed at 120 min at suboptimal conditions in acetate buffer than in chloride.

Table II: Effect of Magnesium Ion Concentration on Stimulation of Single-Stranded DNA-Dependent ATPase Activity of RecA Protein by SSB Protein[a]

	magnesium ion concn (mM)			
anion	1	2	3	4
chloride	−	−	+	+
acetate	−	+	+	+

[a] SSB protein was added to the standard assay mixture 1 min after recA protein. Stimulation observed (+); inhibition observed (−).

Kowalczykowski and Krupp (1986) have shown that, at concentrations of magnesium chloride greater than or equal to 4 mM, SSB protein will stimulate the single-stranded DNA-dependent ATPase activity of recA protein. At 1 mM magnesium chloride, however, SSB protein is inhibitory. They demonstrated that SSB protein is able to displace recA protein from single-stranded DNA at the low magnesium ion concentration but that at high magnesium concentrations recA protein is resistant to this displacement and is able to form a recA protein saturated presynaptic complex with the single-stranded DNA. Thus, the differences seen in Figure 6 may be the result of a difference in the magnesium concentration dependence of the formation of presynaptic complexes in acetate vs. chloride buffer. To test this possibility, the effect of SSB protein on the ATPase activity of recA protein was assayed. Table II summarizes our data and shows that, at 2 mM magnesium, SSB protein has an inhibitory effect on the single-stranded DNA-dependent ATPase activity in chloride buffer but a stimulatory one in acetate buffer, while at 1 mM magnesium, SSB protein has an inhibitory effect in both buffers. These results parallel the magnesium dependence of strand exchange as seen in Figure 6 and suggest that the differences in magnesium concentration dependence between chloride and acetate buffers may be a consequence of the ability of recA protein to form a presynaptic complex in the presence of SSB protein.

Aggregation Properties of RecA Protein Molecules Appear To Be Equivalent at 37 °C Whether in Chloride or Acetate Buffer. Since neither the single-stranded DNA binding affinity nor the single-stranded DNA-dependent ATPase activity directly correlates with the inhibition of strand exchange activity in increasing salt concentrations, the relationship of aggregation to strand exchange was investigated. It is known that magnesium will induce the aggregation and precipitation of recA protein and that, at 25 °C, this aggregation is inhibited by concentrations of sodium chloride that are similar to those inhibitory to strand exchange (Cotterill et al., 1982). Thus, acetate anions may also have an effect on a protein–protein aggregation step.

In an attempt to correlate the degree of recA protein aggregation with its ability to catalyze strand exchange, increments of magnesium salts were added to a cuvette containing recA protein, and the resulting turbidity was measured via light scattering. This aggregation reaction was studied under conditions used in the strand exchange reaction (i.e., at 37 °C and in the presence of single-stranded DNA). Under these conditions, no difference was observed between aggregation in chloride and acetate buffers (Figure 7). Magnesium titrations were also performed with 1 mM ATP and single-stranded and/or duplex DNA in the buffer (not shown). In the presence of ATP, aggregation is reduced approximately 10-fold, and the onset of aggregation, observed at about 6 mM magnesium in the absence of ATP (Figure 7), is shifted to 1 or 2 mM magnesium in both chloride and acetate buffers. In addition, the titration curves are virtually superimposable both in the presence (not shown) and in the absence of SSB protein.

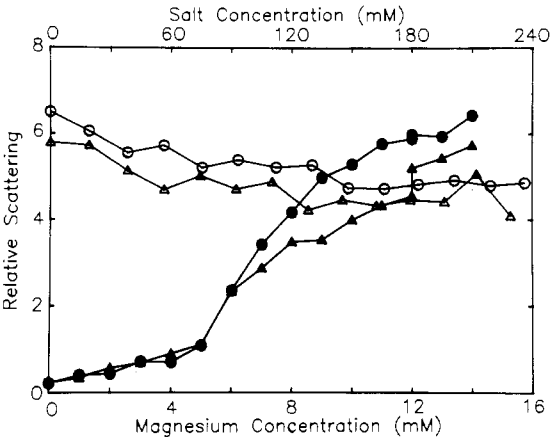

FIGURE 7: Aggregation of recA protein as a function of magnesium ion and salt concentration at 37 °C. Titrants: magnesium chloride (●); magnesium acetate (▲); sodium chloride (○); sodium acetate (△). Standard aggregation assay conditions in the absence of ATP were used. Magnesium was titrated into buffer containing 6.0 μM recA protein and 9.9 μM single-stranded DNA to obtain the data on the formation of aggregates. To obtain the data on the dissociation of these aggregates, salt was titrated directly into the cuvette containing the magnesium-induced aggregates created during the magnesium titration.

No significant differences in magnesium-induced aggregation were ever observed between chloride and acetate buffers.

In addition, salt titrations were performed in which aliquots of either sodium chloride or sodium acetate were added to aggregates formed above. In this case, dissociation was measured as a decrease in turbidity of the solution (Figure 7). Again, no difference was observed in the pattern of aggregate dissociation between chloride or acetate buffers in both the presence and absence of ATP, single-stranded DNA, and duplex DNA. Complexes are stable up to 250 mM sodium chloride or sodium acetate, suggesting that the step affected by salt in strand exchange is not the aggregation property being measured in these turbidity assays.

To ensure that our method for measuring aggregation was comparable to those already published, we performed identical magnesium and salt titrations under the conditions of Cotterill and Fersht (1983) (i.e., at 25 °C). Our results in chloride buffer (not shown) paralleled those of Cotterill and Fersht (1983) but were significantly different from those we obtained at 37 °C (Figure 7). At 25 °C in chloride buffer, a large increase in aggregation occurred at 10 mM magnesium chloride, while in acetate buffer, this large increase occurred at 7–8 mM magnesium acetate. These complexes were dissociated in either buffer by 50 mM sodium salt.

These results indicate that, under conditions in which in vitro strand exchange is performed, the degree of aggregation cannot explain the differences between chloride and acetate buffers either in the rate of product formation or in the salt sensitivity of the strand exchange reaction.

Acetate Buffer Increases the Rate of Hydrolysis and Decreases the Lag Observed in Duplex DNA-Dependent ATPase Activity. Although the magnesium dependence of strand exchange activity appears to be related to the ability of SSB protein to stimulate the single-stranded DNA-dependent ATPase activity of recA protein, it is clear that this property of recA protein does not explain the salt sensitivity of the strand exchange reaction since SSB protein will stimulate the ATPase activity up to 300 mM sodium chloride (Kowalczykowski & Krupp, 1986). Also, since neither single-stranded DNA binding nor protein aggregation properties seemed to be responsible for the difference in the salt sensitivity of strand

exchange in chloride and acetate buffers, the duplex DNA-stimulated ATPase activity of recA protein was examined.

Kowalczykowski (1985) and Kowalczykowski et al. (submitted for publication) observed that the duplex DNA-dependent ATPase activity of recA protein exhibits an initial lag in ATP hydrolysis which is not observed in single-stranded DNA-dependent ATPase assays. This lag was shown to represent the rate-limiting step in the duplex DNA-dependent ATPase activity of recA protein and was proposed to be involved in the local denaturation of the duplex DNA. To determine whether this property of recA protein was related to the salt sensitivity of strand exchange activity, the duplex DNA-dependent ATPase activity was investigated in both chloride and acetate buffers at various salt concentrations. As shown in Figure 8, a lag exists before the onset of maximal ATP hydrolysis. This lag is decreased and the terminal rate of hydrolysis is increased in the presence of acetate buffer as compared to the data obtained in chloride buffer. This ATPase activity is also very salt sensitive, and Figure 8 shows that, in identical concentrations of sodium chloride in chloride buffer or sodium acetate in acetate buffer, the lag is shorter and the rate is greater in the presence of acetate ions.

To ensure that the presence of single-stranded DNA had no effect on the duplex DNA-dependent ATPase activity during the strand exchange reaction, the total ATPase activity of single- and double-stranded DNA, together under strand exchange assay conditions, was determined in the presence and absence of SSB protein (not shown). The final ATPase activity was always less than or equal to the additive total of the single-stranded and duplex DNA-dependent ATPase activities, demonstrating that, under these conditions, single-stranded DNA does not stimulate the duplex DNA-dependent reaction.

To determine whether a relationship exists between duplex-dependent ATPase activity and the quantity of product formed during strand exchange, the amount of ATP hydrolyzed after 30 min was calculated[2] and plotted as a function of the amount of product formed in 30 min (from the data from which Table I is derived) at each of the various salt concentrations in both chloride and acetate buffers. As Figure 9 shows, there is a direct, linear relationship between the number of ATP molecules hydrolyzed and the amount of product formed. Since this relationship is linear, the slope of the line in Figure 9 defines the number of ATP molecules hydrolyzed (in the duplex DNA-dependent reaction only) per nucleotide of product formed after 30 min of strand exchange. The slope of this line is 4.9 ± 0.1 mol of ATP hydrolyzed per mole of nucleotides incorporated.

These results demonstrate that the sensitivity of strand exchange to the ionic strength of the buffer linearly parallels the recA protein duplex DNA-dependent ATPase activity and suggest that the rate-limiting steps in strand exchange product formation and in duplex DNA-dependent ATPase activity are related.

Relationship between Duplex DNA-Dependent ATPase Activity and Strand Exchange. Kowalczykowski et al. (submitted for publication) have postulated that the rate-limiting step in duplex DNA-dependent ATPase activity is the local denaturation of the duplex DNA. Cox and Lehman (1981) have shown that the rate-limiting step in strand exchange is branch migration. Since our data demonstrate a direct parallel between the amount of product formed during strand exchange and the duplex DNA-dependent ATPase activity, it is logical to conclude that local denaturation of the DNA, as measured by duplex-dependent ATPase activity, is the rate-limiting event during the branch migration step of the strand exchange process.

Assuming this conclusion to be correct, we have determined that the number of ATP molecules hydrolyzed per base pair exchanged is approximately five (Figure 9). However, the duplex DNA-dependent ATP hydrolysis rate accelerates with time (see Figure 8) due to the fact that once recA protein has invaded the duplex DNA, it is capable of undergoing many rounds of ATP hydrolysis (Kowalczykowski et al., submitted for publication). Presumably, only the first ATP hydrolysis event would be productive in branch migration with subsequent events being nonproductive with regard to product formation. In addition, since recA protein molecules remain bound to the duplex DNA product molecules (Soltis & Lehman, 1983), ATP will continue to be hydrolyzed by the bound recA protein even though strand exchange has already occurred. Therefore, to determine the limiting value of the number of ATP molecules hydrolyzed per nucleotide exchanged, data such as those shown in Figure 9 must be extrapolated to zero time. This extrapolation was accomplished by determining the amount of strand exchange product formed at early times from data such as that shown in Figure 1. For each of these time points, the corresponding amount of ATP hydrolyzed in a parallel duplex DNA-dependent reaction was determined from Figure 8. From these two values, the number of ATP molecules hydrolyzed per nucleotide of product incorporated was determined and plotted as a function of time in Figure 10. As expected, the apparent efficiency of ATP utilization, as defined by the amount of ATP hydrolyzed per nucleotide of product formed, decreases with increasing time due to an increasing amount of nonproductive ATP hydrolysis over time. Extrapolation of this plot to zero time yields a value of 0.75 ± 0.25 ATP molecule hydrolyzed per nucleotide of product incorporated. This number represents a limiting value for the number of ATP molecules that are hydrolyzed during the branch migration step of the strand exchange reaction.

Discussion

We have demonstrated that the initial rate of the strand exchange activity of recA protein is approximately 3–4-fold greater when assayed in a buffer containing acetate rather than chloride ions. These effects are seen in agarose gel assays, where a 100% product yield occurs in acetate buffer after approximately 30–45 min of reaction, while in chloride buffer, this yield is not observed until 90–120 min (Figure 1). These results are paralleled by those of the D-loop assays in which the initial rate in acetate buffer is twice that in chloride buffer (Figure 3). In addition, we have demonstrated that, contrary to expectations, recA protein catalyzed strand exchange involving linear duplex and circular single-stranded DNA molecules is irreversible. Strand exchange involving this gapped circular duplex and linear single-stranded DNA is not observed.

The fact that there is a difference in the rate of strand exchange by recA protein resulting from the presence of acetate or chloride ions implies that at least one type of ionic interaction is affected. In an attempt to correlate the effects of salts and the different anions on various physical and enzymatic properties of recA protein with the ability of recA protein to catalyze strand exchange, we examined the effects of sodium and magnesium salts on single-stranded DNA-dependent ATPase activity, protein aggregation, the formation

[2] The ATP hydrolysis data are derived from Figure 8, except for 0 and 50 mM sodium acetate and 0 mM sodium chloride for which longer time course experiments with a 2-fold greater amount of NADH were required (not shown). Under our conditions, a decrease of 1 ODU is equivalent to the hydrolysis of 0.16 mM ATP.

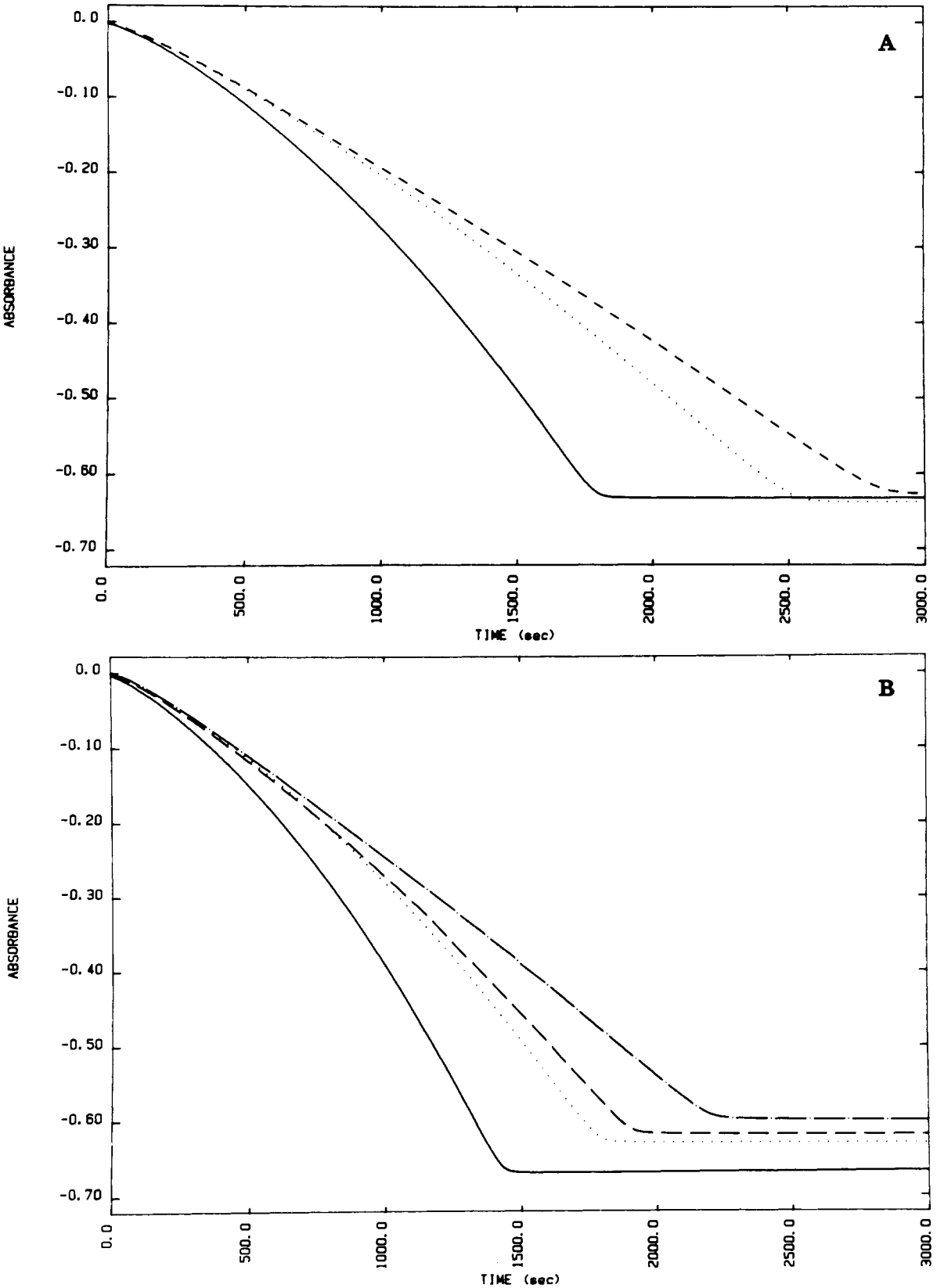

FIGURE 8: Effect of salt concentration on duplex DNA-dependent ATPase activity. (A) Effect of sodium chloride on duplex DNA-dependent ATPase activity in chloride buffer. (B) Effect of sodium acetate on duplex DNA-dependent ATPase activity in acetate buffer: 0 mM (solid line); 50 mM (dotted line); 100 mM (dashed line); 200 mM (dash–dot line). Standard ATPase assay conditions were used. The various salts were added directly to the buffer before the addition of any other components. A decrease of 1 ODU represents the hydrolysis of 0.16 mM ATP.

of a presynaptic complex in the presence and absence of SSB protein, and duplex DNA-dependent ATPase activity. From these comparisons, it became evident that both the magnesium dependence and the sodium salt dependence of strand exchange could not be explained by a single property of recA protein. Thus, at least two different properties of this protein must be responsible for the inhibitory effects observed at low magnesium and high salt concentrations.

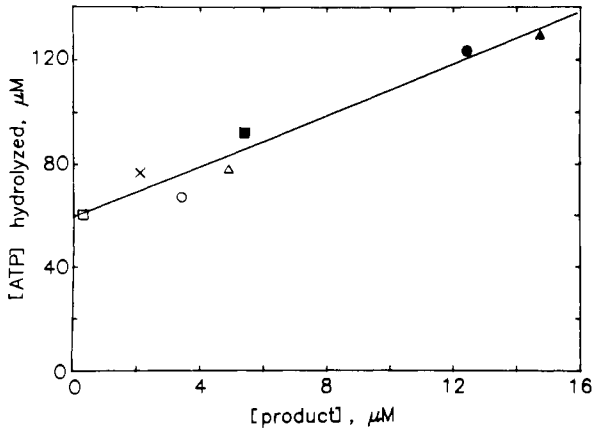

FIGURE 9: Relationship between ATP hydrolyzed in the duplex DNA-dependent reaction and amount of product formed in the strand exchange reaction. Points are taken at 0, 50, 100, and 200 mM sodium chloride and sodium acetate concentrations after 30 min of reaction in each assay. The data were derived from the experiments summarized in Table I and Figure 8 as described in the text. Open symbols represent sodium chloride data, and closed symbols represent sodium acetate data: 0 mM (△); 50 mM (○); 100 mM (□); 200 mM sodium acetate (×).

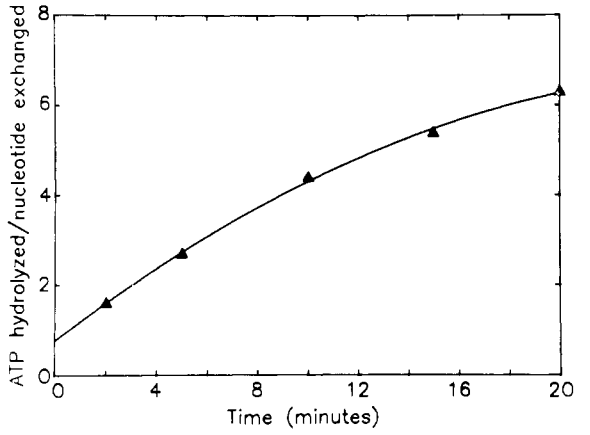

FIGURE 10: Number of ATP molecules hydrolyzed per base pair of product formed during strand exchange. The data are derived from Figures 1 and 8, as described in the text.

RecA protein binds to single-stranded DNA with greater affinity in the presence of acetate ions than in the presence of chloride ions (Menetski & Kowalczykowski, 1985). The relatively unchanged level of recA protein catalyzed ATPase activity observed in salt concentrations up to 400 mM in acetate buffer as opposed to decreasing levels of activity under the same conditions in chloride buffer is consistent with the greater affinity that recA protein has for single-stranded DNA in acetate buffer. The concentration of salt required to dissociate these single-stranded DNA–recA protein complexes (840 mM sodium acetate in acetate buffer or 280 mM sodium chloride in chloride buffer) and inhibit the single-stranded DNA-dependent ATPase activity is, however, 3–5-fold higher than that required to inhibit strand exchange in either case. Consequently, strand exchange activity is abolished long before dissociation of recA protein–single-stranded DNA complexes occurs. Therefore, neither the single-stranded DNA binding affinity nor the single-stranded DNA-dependent ATPase activity of recA protein appears to limit its ability to catalyze strand exchange at the higher salt concentrations. In addition, since recA protein is capable of both binding to single-stranded DNA and hydrolyzing ATP at low magnesium concentrations (e.g., 1 mM magnesium chloride), these properties of recA protein are also unable to explain the magnesium dependence of strand exchange.

Although the single-stranded DNA-dependent ATPase activity of recA protein does not appear to correlate with the inhibition of strand exchange activity, the properties of this ATPase activity are altered when SSB protein is present. Kowalczykowski et al. (1986) and Kowalczykowski and Krupp (1986) have shown that the magnesium concentration greatly affects the ability of recA protein to become resistant to dissociation from single-stranded DNA by SSB protein. Single-stranded DNA-dependent ATPase assays in the presence of SSB protein indicate that recA protein is resistant to displacement by SSB protein at a lower magnesium concentration in acetate than in chloride buffer (Table II) and that the magnesium concentrations at which stimulation by SSB protein occurs coincide exactly with the minimum magnesium concentration required for strand exchange in either chloride or acetate buffers (Figure 6). Thus, the magnesium concentration requirement in strand exchange appears to be a consequence of the ability of recA protein to resist desplacement by SSB protein and so form a completely saturated presynaptic complex. However, the salt sensitivity of the single-stranded ATPase in the presence or absence of SSB protein does not correlate with the effect of salt concentration on the formation of product in strand exchange, indicating that neither the formation nor the stability of the presynaptic complex is the major component affected by salt.

We have shown that both the yield of strand exchange and the ability of SSB protein to stimulate the single-stranded DNA-dependent ATPase activity in both acetate and chloride buffers increase with increasing magnesium concentration. Since aggregation is a property of recA protein known to be dependent on magnesium concentration (Cotterill et al., 1982), we investigated whether the degree of aggregation could be correlated with the ability of recA protein to catalyze strand exchange. At 37 °C and in the presence or absence of ATP, there is no apparent difference in either aggregate formation or aggregate dissociation between chloride and acetate buffers with regard to either magnesium or salt concentration. Therefore, the degree of aggregation of recA protein is apparently not responsible for the salt sensitivity of the strand exchange reaction.

Since the effect of increasing salt concentration on either single-stranded DNA binding or protein aggregation could not explain the salt inhibition of recA protein catalyzed strand exchange, the duplex DNA-dependent ATPase properties of recA protein were examined. Of all the physical and enzymatic properties investigated, the duplex DNA-dependent ATPase activity shows the best correlation with strand exchange regarding both sodium chloride and sodium acetate salt sensitivity, suggesting that the duplex DNA-dependent ATPase activity plays an important rate-limiting role in strand exchange. Although the exact roles of single- or double-stranded DNA-dependent ATPase activity in the catalysis of strand exchange have not yet been clarified, Kowalczykowski et al. (submitted for publication) have proposed that the duplex DNA-dependent ATPase is a measure of the local denaturation of the duplex DNA. We have shown that this duplex ATPase activity correlates very closely with the ability of recA protein to catalyze strand exchange and must therefore be involved in the rate-limiting step of strand exchange. Since Cox and Lehman (1981) have demonstrated that branch migration is the rate-limiting step in strand exchange, a logical conclusion is that the duplex DNA-dependent ATPase activity is involved in the opening of the duplex during branch migration.

Assuming that the duplex-dependent ATPase activity is involved in the denaturation of the duplex DNA during branch migration, we have calculated the number of ATP molecules hydrolyzed per nucleotide assimilated. This value is 0.75 and represents the lowest estimate of the number of ATP molecules hydrolyzed per nucleotide assimilated reported in the literature to date. By comparison, Cox et al. (1983) have shown that, under standard strand exchange conditions, 100–200 ATP molecules are hydrolyzed per nucleotide assimilated and that, in the presence of low levels of ADP, this estimate is reduced to 16.

Our determination of the energetic cost of branch migration demonstrates that this process may be much more efficient than previous estimates would have led one to believe, when the mostly nonproductive ATP hydrolysis due to the single-stranded DNA-dependent ATPase activity is removed from the estimate. In principle, the maximum number of base pairs that can be opened or exchanged per ATP molecule hydrolyzed would be equal to the duplex DNA binding site size of a recA protein monomer. This value is approximately five base pairs (Kowalczykowski et al., submitted for publication). This estimate, in fact, may be too large since, under our conditions, the free energy derived from ATP hydrolysis could only "open" two to four base pairs, depending on nucleotide composition. Thus, our value of 0.75 ATP molecule hydrolyzed per base pair exchanged is within, at most, a factor of 4 and possibly less than a factor of 2 different from the maximum theoretical value. In addition, our value of 0.75 ATP molecule hydrolyzed per nucleotide exchanged must still represent a slight overestimate since at early time points in the strand exchange assay, there are DNA molecules in intermediate stages of branch migration and consequently not visible on the gel as final product molecules.

Thus, the results of the biochemical studies presented here suggest that two properties of recA protein important to the strand exchange reaction are the ability of recA protein to displace SSB protein from single-stranded DNA and the duplex DNA-stimulated ATPase activity. At low magnesium concentrations, the former process becomes limiting in strand exchange whereas, at high salt concentrations, the latter becomes limiting. Consistent with these observations are the properties of recA142 protein, a defective mutant of recA protein that is unable to catalyze the strand exchange reaction (Burk and Kowalczykowski, unpublished observation). The mutant protein appears to be competent in single-stranded DNA binding and ATPase activity, and its aggregation properties are identical with those of recA protein. The mutant protein is, however, unable to displace SSB protein from single-stranded DNA and is completely defective in duplex DNA-dependent ATPase activity—the two properties which explain the differences in the ability of recA protein to catalyze strand exchange in chloride and acetate buffers.

The apparent irreversibility of recA protein catalyzed strand exchange involving linear duplex and circular single strand is puzzling, and the explanation is not readily obvious. This irreversibility is not due to an inhibition of the reverse reaction by protein molecules bound irreversibly to the product DNA molecules since the gapped circular and linear single-stranded substrates, extracted with phenol to remove all proteins, are not active substrates in the reverse reaction to re-form the original linear duplex and circular single-stranded DNA substrates.

The fact that renaturation of the DNA substrate molecules in the absence of proteins results in an equimolar mixture of the product molecules indicates that the free energy of formation of the products is equal to that of the substrates and that the irreversibility is not the result of intrinsic differences in the stability of the DNA product molecules as compared to the stability of the substrate molecules. Therefore, the presence of recA protein or SSB protein must somehow perturb this equilibrium so as to favor product formation. Because it is known that, in the presence of single-stranded DNA, recA protein will form linear aggregates of protein–DNA complexes (Dunn et al., 1982; Flory & Radding, 1982), it is possible that the free energy of this linear aggregate formation is favored with linear single-stranded DNA as compared to circular single-stranded DNA. Since one of the products of the strand exchange reaction is a linear single-stranded DNA molecule, the equilibrium position of this reaction may be pulled toward product formation. The fact that no such bias is observed in a reaction involving identical substrates except in a linear duplex and linear single-stranded DNA form tends to support this interpretation. Alternatively, the reverse reaction may be prevented for kinetic reasons which have not yet be determined. Register and Griffith (1985) observed that, in the presence of recA and SSB proteins, linear single-stranded DNA, but not circular single-stranded DNA, contains a short segment at one end which is complexed with SSB protein. Perhaps the inability of recA protein to displace SSB protein from these ends constitutes a block to its ability to catalyze strand exchange with these substrates.

Finally, the question of the physiological relevance of acetate- vs. chloride-based buffers, can be raised. In a review of ionic effects on protein–nucleic acid interactions, Record et al. (1986) state that the major free anions in an *Escherichia coli* cell appear to be chloride (Schultz et al., 1962) and glutamate, with glutamate being the dominant species under some conditions (i.e., high external osmolarity) (Munro et al., 1972; Measures, 1975). Studies in this lab have shown that, in the presence of glutamate, recA protein binds so tightly to single-stranded DNA that dissociation does not occur even in the presence of 1.2 M sodium glutamate (Menetski et al., unpublished results). Therefore, a buffer containing acetate ions, in which recA protein binds to single-stranded DNA more tightly than in chloride buffer but less tightly than in glutamate buffer, may more closely simulate the environment inside the cell.

In summary, we have shown that acetate-based buffers have a dramatic effect on the rate of the strand exchange reaction. This observation should be of practical importance in studies of other recA protein catalyzed reactions and in optimizing the yield of product DNA molecules. The molecular basis of this enhancement appears to be primarily related to the duplex DNA-dependent ATPase activity of recA protein. The salt sensitivity of strand exchange correlates very well with the salt sensitivity of duplex DNA-dependent ATPase activity, suggesting that their rate-limiting steps are related. If, as we have deduced, the rate-limiting step during branch migration is the denaturation of the duplex DNA, the number of ATP molecules hydrolyzed per base assimilated is equal to 0.75 or less. The magnesium dependence of the strand exchange reaction appears, in part, to be related to the ability of recA protein to become resistant to displacement by SSB protein during presynaptic complex formation.

Registry No. ATPase, 9000-83-3; Mg, 7439-95-4; Cl^-, 16887-00-6; AcOH, 64-19-7.

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