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Characterization of the Adenosinetriphosphatase Activity of the *Escherichia coli* RecBCD Enzyme: Relationship of ATP Hydrolysis to the Unwinding of Duplex DNA†

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ABSTRACT: We find that the rate of dsDNA-dependent ATPase activity is biphasic, with a fast component which represents the unwinding of the dsDNA and a slow component which results from the ssDNA-dependent ATPase activity of recBCD enzyme. Comparison of the ATPase and helicase activities permits evaluation of the efficiency of ATP hydrolysis during unwinding. This efficiency can be calculated from the maximum rates of ATPase and helicase activities and is found to range between 2.0 and 3.0 ATP molecules hydrolyzed per base pair of DNA unwound. The number of ATP molecules hydrolyzed per base pair unwound is not altered by temperature but does increase at low concentrations of DNA and high concentrations of sodium chloride and magnesium acetate. The apparent K_m values for the DNA and ATP substrates of recBCD enzyme dsDNA-dependent ATPase activity at 25 °C were determined to be 0.13 nM DNA molecules and 85 μM ATP, respectively. The observed k_{cat} value is approximately 45 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$. If this rate is corrected for the measured stoichiometry of recBCD enzyme binding to dsDNA, the k_{cat} for ATPase activity corresponds to an ATP hydrolysis rate of approximately 740 ATP molecules s^{-1} (functional recBCD complex)$^{-1}$ at 25 °C.

The recBCD enzyme is composed of three nonindentical subunits which together catalyze five distinct biochemical reactions: single-stranded DNA (ssDNA)[1] exo- and endonuclease activities, dsDNA exonuclease activity, helicase activity, and a DNA-dependent ATPase activity [for reviews, see Telander-Muskavitch and Linn (1981) and Taylor (1988)]. We have extensively characterized the helicase activity of recBCD enzyme (Roman & Kowalczykowski, 1989) and now wish to examine the DNA-dependent ATPase activity and its relationship to the unwinding of dsDNA.

Characterization of the ATPase activity of recBCD enzyme has, to date, been performed under conditions such that it could be studied as a component of recBCD enzyme nuclease activities. Eichler and Lehman (1977) and Goldmark and Linn (1972) showed that the degradation of dsDNA by recBCD enzyme requires ATP and exhibits an optimum at 20 μM ATP. Under these conditions, the products of the nuclease reaction are single-stranded oligonucleotides three to eight bases long. They have also demonstrated that the extent of exonuclease activity is inhibited at high (>200 μM) concentrations of ATP where the product lengths range from 135 to 1400 nucleotides (Mackay & Linn, 1976) while the initial rate of nuclease activity remains unaffected. This difference in products at low and high ATP concentrations led Mackay and Linn (1976) to propose the explanation that recBCD enzyme nicks the DNA at a constant rate but moves more quickly through the dsDNA at higher ATP concentration, thus releasing larger product molecules. We have shown (Roman & Kowalczykowski, 1989) that recBCD enzyme does, indeed,

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[1] Abbreviations: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB protein, *E. coli* ssDNA binding protein; RF, replicative form; SDS, sodium dodecyl sulfate; DTT, diithiothreitol; ATP, adenosine triphosphate; EDTA, ethylenediaminetetraacetic acid; NADH, nicotinamide adenine dinucleotide (reduced).

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unwind DNA at a faster rate as ATP concentration is raised and that the apparent K_m value for ATP in the unwinding reaction is 0.13 mM.

Karu and Linn (1972) demonstrated that the ATPase activity could be uncoupled from the dsDNA exonuclease activity by introducing cross-links into the DNA. Nuclease activity was inhibited completely (as presumably was the unwinding activity) while ATPase activity was unaffected. They postulated that recBCD enzyme bound to and "stalled" on the DNA, unable to degrade the DNA but continuously hydrolyzing ATP molecules. Rosamond et al. (1979) also confirmed that ATPase activity was uncoupled from nuclease activity and proposed that it is more closely related to helicase activity. They demonstrated that ATPase activity is totally unaffected by the presence of high ATP concentrations, SSB protein, and the divalent calcium ion, conditions which favor unwinding but inhibit nuclease activity almost completely. In this paper, we characterize the ATPase activity of recBCD enzyme and compare it to the unwinding activity described in the previous paper (Roman & Kowalczykowski, 1989), thus allowing us to determine how closely related this ATPase activity is to the helicase activity of recBCD enzyme under a variety of conditions.

Materials and Methods

All DNA and protein isolations are as described in the preceding paper (Roman & Kowalczykowski, 1989). M13 mp7 ssDNA is linearized by digestion with *Eco*RI to remove the hairpin formed by the mp7 cloning insert.

Lactate dehydrogenase and pyruvate kinase were purchased from Sigma as ammonium sulfate suspensions. These suspensions were spun down, and the pellet was resuspended in reaction buffer. ATP was purchased from Pharmacia P-L Biochemicals and dissolved as a concentrated stock at pH 7.5. The ATP concentration was determined by using an extinction coefficient of 15.4×10^3 cm^{-1} M^{-1}.

ATPase Assay. ATPase activity was measured according to the procedure described by Kreuzer and Jongeneel (1983). 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 with a Hewlett Packard 8450 spectrophotometer. The raw data shown (i.e., Figure 1) represent the depletion of NADH, which is directly proportional to the amount of ATP hydrolyzed. A decrease of 1 optical density unit at 340 nm is equivalent to the hydrolysis of 0.16 mM ATP. The initial rate of ATP hydrolysis was calculated by determining the slope of a tangent drawn to the linear data after the start of the reaction. The measured rate of ATP hydrolysis is independent of the concentrations of the coupling reagents present; this system can convert ADP to ATP at least 10-fold faster than recBCD enzyme can hydrolyze ATP, as measured by the conversion of ADP (ranging from 10 to 100 μM) added directly to the reaction (not shown). For more details on this assay, see Kowalczykowski and Krupp (1987).

The assays were performed at 25 °C and begun with the addition of dsDNA to a cuvette (0.3 or 0.5 mL) containing all other components. The reaction buffer contained 25 mM Tris–acetate (pH 7.5), 1.0 mM magnesium acetate, 1.0 mM ATP, 1.0 mM dithiothreitol, 1.5 mM phosphoenolpyruvate, 100 μg/mL NADH, and approximately 20 units/mL each of lactate dehydrogenase and pyruvate kinase. The standard reaction mixture contained enough SSB protein to saturate an amount of ssDNA equivalent to the amount of dsDNA in the reactions 1.5-fold, assuming a site size of 15 nucleotides per monomer, unless otherwise indicated.

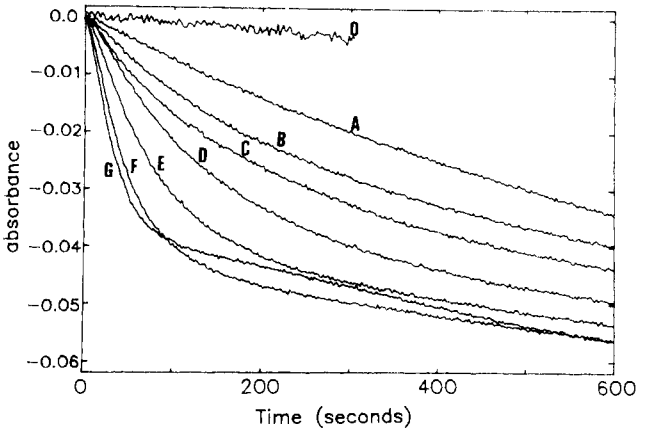

FIGURE 1: Effect of recBCD enzyme concentration on its dsDNA-dependent ATPase activity. Lines A–G: 0.43, 0.85, 1.4, 2.1, 4.2, 8.4, and 21 nM recBCD enzyme, respectively. Line 0 is 0 nM recBCD enzyme. The concentrations of dsDNA and SSB protein were 0.35 nM and 1.0 μM, respectively. Standard buffer conditions were used. A decrease in absorbance of 1 OD unit is equivalent to the hydrolysis of 0.16 mM ATP.

Results

The Hydrolysis of ATP Is Related to the Unwinding of dsDNA. The data shown in Figure 1 represent the typical raw data generated by the ATPase assay at various concentrations of recBCD enzyme. Classic enzymatic behavior is observed in that increasing the enzyme concentration results in an increase in the initial rate. The linear dsDNA-dependent ATPase activity in the presence of SSB protein is biphasic, consisting of an initial fast component and a subsequent slower component, an unexpected result as there was no indication of such behavior in the literature. These curves show a striking resemblance to the time course of DNA unwinding (see Figure 1, Roman & Kowalczykowski, 1989) under the same conditions. The initial, fast component of the ATPase profile temporally coincides exactly with unwinding of dsDNA under the same conditions. For example, lines drawn tangent to the initial and plateau regions of line F (Figure 1) intersect at about 75 s. This same result is obtained if helicase data such as those shown in Figure 1 of the previous paper (Roman & Kowalczykowski, 1989), but under the same conditions, are used (data not shown). No reaction occurs in the absence of recBCD enzyme (Figure 1, line 0), and a small amount of hydrolysis is observed in the absence of dsDNA [5 ± 1 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$; Figure 2, line C]. Whether this small amount of DNA-independent activity is due to an intrinsic ability of recBCD enzyme to hydrolyze a small amount of ATP in a DNA-independent fashion or to trace amounts of some contaminant is unknown.

With a substrate that does not support helicase activity (i.e., supercoiled M13 RF DNA), or with ssDNA, the initial ATP hydrolysis rate is slower and no longer biphasic (Figure 2). For both the single-stranded (line A) and the supercoiled (line B) DNA in the presence of SSB protein, there is little ATP hydrolysis [4 ± 1 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$, which is essentially zero when the DNA-independent rate is subtracted out]. These results are consistent with the observation that supercoiled DNA is not a substrate for DNA unwinding (Taylor & Smith, 1980; Roman & Kowalczykowski, 1989) and confirm the results of Mackay and Linn (1976), who have shown that SSB protein will completely inhibit single-stranded or supercoiled DNA-dependent ATP hydrolysis by recBCD enzyme.

For comparison, Figure 2 also shows the linear dsDNA-dependent hydrolysis of ATP in the presence of SSB protein

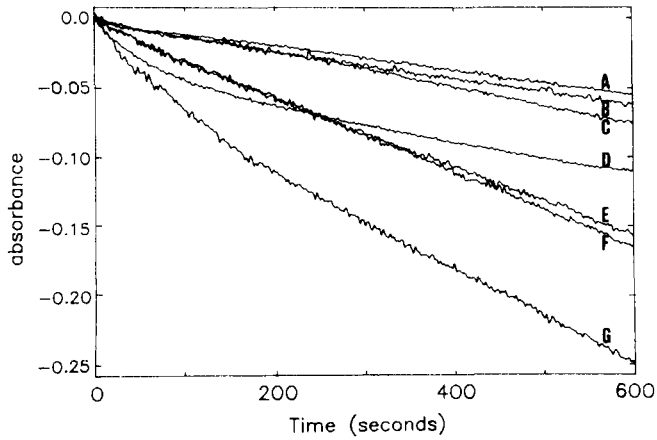

FIGURE 2: Hydrolysis of ATP by recBCD enzyme. Lines A–G: (A) 0.70 nM linear ssDNA molecules with SSB protein present; (B) 0.35 nM supercoiled RF DNA molecules with SSB protein present; (C) no DNA with SSB protein present; (D) 0.35 nM dsDNA molecules with SSB protein present; (E) 0.35 nM supercoiled RF DNA without SSB protein; (F) 0.70 nM linear ssDNA molecules without SSB protein; (G) 0.35 nM dsDNA molecules without SSB protein. The concentration of recBCD enzyme in these experiments was 4.2 nM. Where present, the concentration of SSB protein was 1 μM. Standard buffer conditions were used. A decrease in absorbance of 1 OD unit is equivalent to the hydrolysis of 0.16 mM ATP.

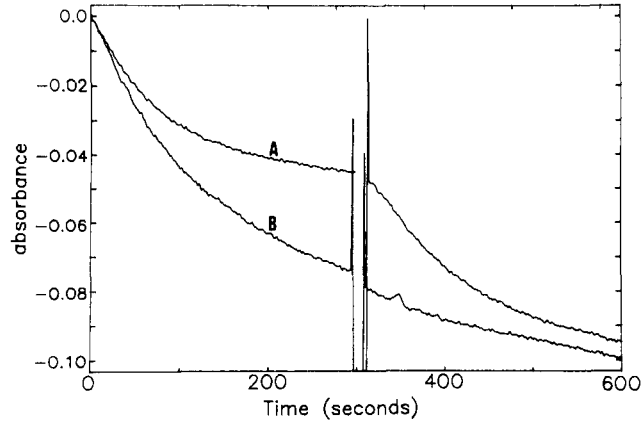

FIGURE 3: Effect of additional DNA or enzyme on the ATPase activity of recBCD enzyme. Line A, 0.35 nM dsDNA molecules added to reaction plateau containing 0.35 nM dsDNA molecules; line B, 4.2 nM recBCD enzyme added to reaction plateau containing 0.70 nM dsDNA molecules. Initial concentration of recBCD enzyme in both reactions was 4.2 nM. Standard buffer conditions and amounts of SSB protein were used. A decrease in absorbance of 1 OD unit is equivalent to the hydrolysis of 0.16 mM ATP.

(line D). The initial rate of the fast component is 29 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$. The rate of the slow component is 3 μM ATP s^{-1} μM^{-1}, which is zero when the DNA-independent rate is removed. Since complete DNA unwinding occurs during the time frame of the fast component of the ATP hydrolysis reaction, it appears that only the fast component of the dsDNA ATPase activity is related to unwinding of the dsDNA. The slow component observed in the presence of SSB protein is unrelated to DNA unwinding and reflects DNA-independent hydrolysis (compare line D to line A).

When SSB protein is omitted from the ATPase assays, the time course observed using linear dsDNA is still biphasic, although the distinction between the two phases is blurred due to the increased rate of hydrolysis for the slow component (Figure 2, line G). The rate of the fast component [29 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$] is, however, the same whether the reaction is performed in the presence or absence of SSB protein. This indicates that, in contrast to the results of Mackay and Linn (1976), SSB protein has no effect on the initial rate of linear dsDNA-dependent ATPase activity. The rate of the slow component is 8 μM ATP s^{-1} μM^{-1}.

To determine whether the increased rate of ATP hydrolysis observed for the slow phase in the absence of SSB protein is due to stimulation of ATPase activity by ssDNA (resulting from DNA unwinding), the ssDNA-dependent ATPase activity in the absence of SSB protein was examined (Figure 2, line F). The rate of ssDNA-dependent ATP hydrolysis is faster in the absence of SSB protein than in its presence, as is the second, slow component observed in the reaction dependent on dsDNA. In the absence of SSB protein, the rate of linear ssDNA-dependent ATP hydrolysis [6 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$, when DNA independent rate is removed] is linear in time and is similar to the ATP hydrolysis rate obtained for the slow component of the dsDNA-dependent reaction under these conditions. Thus, in the absence of SSB protein, the slow component observed in the presence of linear dsDNA is most likely due to ssDNA-dependent ATP hydrolysis resulting from the formation of ssDNA by recBCD enzyme helicase activity. When SSB protein is present, this ssDNA-dependent ATP hydrolysis is reduced to the background DNA-independent rate.

Also shown in Figure 2 (line E) is the supercoiled DNA-dependent hydrolysis of ATP in the absence of SSB protein. The rate of hydrolysis is 5 μM ATP s^{-1} μM^{-1}, equal to that observed with ssDNA. Thus, supercoiled DNA, which cannot be unwound by recBCD enzyme, is able to support the ATP hydrolysis activity of recBCD enzyme. The rates obtained with single-stranded and supercoiled DNA are very similar, however, perhaps indicating that the supercoiled DNA-dependent hydrolysis is a result of the binding of recBCD enzyme to single-stranded regions of the dsDNA.

To further verify that the initial fast component of the ATP hydrolysis reaction is related to the unwinding of dsDNA by recBCD enzyme, an additional amount of dsDNA, equal to the amount of DNA already present, was added after completion of the first phase. As shown in Figure 3, line A, the additional DNA also causes a biphasic behavior in the ATP hydrolysis rate. Both the rate and extent of this second reaction are exactly equal to those of the first reaction, suggesting that the second aliquot of DNA is supporting additional ATP hydrolysis as it is being unwound. As shown in the preceding paper, recBCD enzyme will indeed initiate on and completely unwind DNA which is added subsequent to DNA it has already unwound (Roman & Kowalczykowski, 1989). If additional enzyme is added after the completion of the first phase of the reaction, biphasic behavior is not induced (Figure 3, line B) which is consistent with the fact that the initial aliquot of recBCD enzyme has completely unwound all of the available DNA substrate, as also shown by direct helicase assays (Roman & Kowalczykowski, 1989).

To determine whether recBCD enzyme exhibits a preference for duplex or ssDNA with regard to ATP hydrolysis in the presence of SSB protein, ssDNA (0.69 nM) was added to a ongoing reaction containing dsDNA (0.35 nM). ATP hydrolysis continued as if the ssDNA was not present (not shown). The same result was obtained when the ssDNA was present before the addition of the dsDNA; the reaction proceeds as if the ssDNA was not present (not shown). Thus, all of the results described above parallel exactly the results obtained with the unwinding assay as characterized by Roman and Kowalczykowski (1989).

Effect of SSB Protein on RecBCD Enzyme DNA-Dependent ATPase Activity. Since the presence or absence of SSB protein has a large impact on the second phase of the ATP

Table I: Initial Rates of ATP Hydrolysis for the dsDNA-Dependent ATPase Activity of RecBCD Enzyme[a]

row	[SSB protein] (μM)	initial rate of fast component [μM ATP s^{-1} (μM recBCD)$^{-1}$]	rate[b] of slow component [μM ATP s^{-1} (μM recBCD)$^{-1}$]
1	0.0	20 ± 2	6 ± 0.6
2	0.2	20 ± 2	7 ± 0.7
3	0.4	17 ± 2	4 ± 0.4
4	0.6	18 ± 2	4 ± 0.4
5	0.8	18 ± 2	4 ± 0.4
6	1.0	18 ± 2	4 ± 0.4

[a] All reactions contained 0.14 nM linear duplex M13 DNA molecules and 2.4 nM recBCD enzyme and were performed at 25 °C. Standard buffer conditions were used. [b] Rates of the slow component of ATP hydrolysis are steady-state rates determined from the final 100 s of the reaction.

hydrolysis reaction and since this protein is present in recBCD enzyme-catalyzed unwinding reactions, the concentration of SSB protein was varied in the presence of 0.14 nM dsDNA molecules to determine optimal conditions in which to perform subsequent ATP hydrolysis reactions. As Table I, rows 1–6, indicates, little difference in the initial rate of ATP hydrolysis is observed in the fast component when SSB protein is present at a concentration 1.5-fold greater than that required to fully saturate an equivalent amount of ssDNA. The steady-state rate of the slow component of ATP hydrolysis as a function of SSB protein concentration is also shown in Table I. The rate of the slow component of ATP hydrolysis is essentially background in the presence of concentrations of SSB protein which are sufficient to saturate the total amount of ssDNA present.

The concentration of SSB protein was also varied in the presence of 1.4 nM dsDNA molecules. No difference was observed at SSB protein concentrations which were 1, 2, or 4 times the amount needed to saturate an equivalent amount of ssDNA (not shown). As a result, the SSB protein concentration to be used in all subsequent experiments was chosen to be 1.5-fold saturating of an amount of ssDNA equivalent to the amount of dsDNA present. This allows for a direct comparison with the unwinding data present in the previous paper (Roman & Kowalczykowski, 1989).

The effect of SSB protein on ssDNA-dependent ATPase activity was also examined. If the amount of SSB protein present was greater than or equal to the amount required to fully saturate the 5 μM ssDNA nucleotides present, the rates of ATP hydrolysis measured were not greater than background (not shown). Thus, the ssDNA-dependent ATPase activity of recBCD enzyme is totally inhibited by SSB protein.

Effect of Substrate Concentration on the DNA-Dependent ATP Hydrolysis Activity of RecBCD Enzyme. The concentrations of the two substrates of recBCD enzyme were varied individually to determine the apparent ATP turnover number (k_{cat}) and apparent K_m values for dsDNA and ATP. As shown in Figure 4, hyperbolic behavior is observed when the initial rate of dsDNA-dependent ATP hydrolysis is plotted against substrate concentration. The apparent k_{cat} and K_m values are shown in Table II. The apparent ATP turnover number is equal to approximately 45 μM ATP molecules hydrolyzed s^{-1} (μM recBCD enzyme)$^{-1}$. When corrected for the stoichiometry of recBCD enzyme binding to dsDNA (Roman & Kowalczykowski, 1989; see below), the actual k_{cat} for ATP hydrolysis is 740 μM ATP s^{-1} (μM functional recBCD complex)$^{-1}$. The apparent K_m value for dsDNA is 0.13 nM DNA molecules or 0.26 nM dsDNA ends. The apparent K_m for ATP is 85 μM ATP.

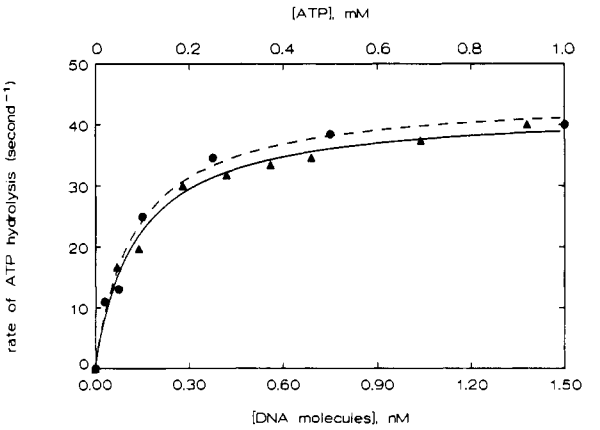

FIGURE 4: Effect of varying substrate concentrations on the ATPase activity of recBCD enzyme: ATP (circles, dashed line); dsDNA (triangles, solid line). The concentration of recBCD enzyme was 2.4 nM and that of dsDNA was 1.7 nM molecules (for ATP concentration dependence). Standard buffer conditions and amounts of SSB protein were used.

Table II: Kinetic Parameters for the ssDNA- and dsDNA-Dependent ATPase Activity of RecBCD Enzyme[a]

assay	substrate	app K_m	app k_{cat}[b]	cor k_{cat}[c]
ATPase	dsDNA	0.13 ± 0.02 (nM molecules)	45 ± 1.3	740 ± 21
	ATP[d]	85 ± 15 (μM)	46 ± 2.2	770 ± 36
	linear ssDNA	1.2 ± 0.6 (μM nucleotides)	2.4 ± 0.5	40 ± 8
	circular ssDNA	0.5 ± 0.3 (μM nucleotides)	3.4 ± 0.6	58 ± 10
helicase[e]	dsDNA	0.6 ± 0.1 (nM molecules)	15 ± 1.2	250 ± 20
	ATP	130 ± 30 (μM)	nc	

[a] All reactions were performed at 25 °C and contained 2.4 nM recBCD enzyme in standard buffer. SSB protein was present only in the reactions involving dsDNA. No SSB protein was present in the reaction to determine kinetic parameters for ssDNA, and DNA-independent ATPase activity was subtracted from data used to determine these parameters. [b] Apparent k_{cat} values are in units of micromolar ATP per second per micromolar recBCD enzyme and micromolar bp per second per micromolar recBCD enzyme for ATPase and helicase activities, respectively. [c] The "corrected k_{cat}" is the "apparent k_{cat}" multiplied by the experimentally observed stoichiometry of recBCD enzyme binding to dsDNA as determined from Figure 4 of the previous paper (Roman & Kowalczykowski, 1989). This stoichiometry is, within experimental error, the same as that obtained in ATPase assays (not shown). However, for consistency, the value from the previous paper (16.5 recBCD enzyme molecules per dsDNA molecule end) was used. [d] Kinetic parameters derived from dsDNA-dependent ATP hydrolysis. [e] From Roman and Kowalczykowski (1989); the apparent K_m value for ATP was calculated by the method of Florini and Vestling (1957). The k_{cat} cannot be calculated by using this method.

The linear ssDNA-dependent ATPase activity of recBCD enzyme was examined in the absence of SSB protein in the same manner and is also shown in Table II. The apparent K_m values for linear and circular ssDNA in the absence of SSB protein are 1.2 ± 0.6 and 0.5 ± 0.3 μM DNA *nucleotides*, respectively. The maximum rates of ATP hydrolysis in the absence of SSB protein on linear and circular ssDNA substrates are 2.4 ± 0.5 and 3.4 ± 0.6 μM ATP molecules hydrolyzed s^{-1} (μM recBCD enzyme)$^{-1}$, respectively, which is about 5–8% of the dsDNA-dependent rate. The fact that the apparent K_m and apparent k_{cat} values for ATP hydrolysis on linear and circular ssDNA molecules are the same, within experimental error, indicates that, unlike recBCD binding to dsDNA, a DNA end is not required and recBCD enzyme is able to bind to the internal nucleotides of the ssDNA molecule. Because a ssDNA end is not the true substrate for recBCD

Table III: Apparent Efficiency of ATP Hydrolysis under Various Conditions[a]

row	variable	initial rate of ATP hydrolysis (s^{-1})	initial rate of unwinding[b] (s^{-1})	app efficiency[c] (ATP/bp)
	[DNA][d] (nM)			
1	0.04	13	0.8	16.3
2	0.07	17	2.2	7.7
3	0.14	21	4.6	4.6
4	0.28	31	5.5	5.6
5	0.43	33	6.5	5.1
6	0.56	35	7.1	4.9
7	0.69	36	9.3	3.9
8	1.04	39	9.4	4.1
9	1.39	42	14.3	2.9
	temp[e] (°C)			
10	16	10	1.6	6.3
11	25	20	3.6	5.6
12	30	24	5.6	4.3
13	37	34	7.4	4.6
	[MgOAc][f] (mM)			
14	1	19	4.4	4.3
15	2	19	5.0	3.8
16	4	19	4.0	4.8
17	6	25	4.2	6.0
18	8	25	4.0	6.3
19	10	25	4.2	6.0

[a] The standard reaction conditions were 2.4 nM recBCD enzyme and 1 mM ATP. The initial rate of ATP hydrolysis is in units of micromolar ATP per second per micromolar recBCD enzyme. The initial rate of unwinding is in the units of micromolar base pairs unwound per second per micromolar recBCD enzyme. [b] Data from Roman and Kowalczykowski (1989). [c] The apparent efficiency is defined as the ratio of the observed ATP hydrolysis rate divided by the observed DNA unwinding rate, at the conditions given. [d] Reactions were performed at 25 °C. The concentration of SSB protein present was enough to saturate an equivalent amount of ssDNA 1.5-fold. [e] Reactions at varying temperatures contained 0.14 nM DNA molecules and 0.4 μM SSB protein. [f] Reactions contained 0.14 nM DNA molecules and 0.4 μM SSB protein and were performed at 25 °C.

enzyme, the apparent K_m values are reported in nucleotide concentration rather than DNA molecule concentration.

Also shown in Table II are the apparent K_m and apparent k_{cat} data derived from the helicase activity of recBCD enzyme (Roman & Kowalczykowski, 1989). Note that the apparent K_m values for ATP generated from both the unwinding and the ATP hydrolysis activities are similar. Apparent K_m values for the dsDNA substrate are, however, definitely different, indicating that the ATPase and helicase activities of recBCD enzyme are not identical with regard to their enzymatic parameters and suggesting that different interactions with the DNA substrate underlie these two activities.

Efficiency of ATP Utilization by RecBCD Enzyme during dsDNA Unwinding. An efficiency of ATP hydrolysis during dsDNA unwinding can be calculated from the apparent k_{cat} values for ATP hydrolysis and dsDNA unwinding shown in Table II. The efficiency in terms of ATP molecules hydrolyzed per base pair unwound, under saturating substrate conditions, is equal to 45 μM ATP molecules s^{-1} (μM recBCD enzyme)$^{-1}$ divided by 15 μM base pairs unwound s^{-1} (μM recBCD enzyme)$^{-1}$ or 3.0. Thus, the unwinding reaction is very efficient with only 3.0 molecules of ATP being hydrolyzed per base pair unwound.

The apparent efficiency of ATP hydrolysis at the various DNA substrate concentrations of Figure 4 was calculated and is shown in Table III, rows 1–9. As dsDNA concentration is decreased, the apparent efficiency of ATP hydrolysis worsens. When the dsDNA concentration is reduced to 0.04 nM molecules, 16 ATP molecules are hydrolyzed per base pair unwound. As dsDNA concentration is increased, the apparent efficiency approaches the limiting value defined by the ratio of k_{cat} values (3.0). This variation in apparent ATP utilization is not unexpected and is due solely to the difference in apparent K_m values for helicase and ATPase activities. Since the apparent K_m values for ATP in both the helicase and ATPase reactions are nearly identical, variation in ATP concentration up to 1 mM has little effect on the observed efficiency of ATP hydrolysis (data not shown).

Effect of Temperature on the ATP Hydrolysis Activity of RecBCD Enzyme. Temperature was varied from 16 to 37 °C to compare the ATPase activity to unwinding activity at various temperatures. The initial rates of ATP hydrolysis and the apparent efficiencies of hydrolysis are shown in Table III, rows 10–13. Since the concentration of DNA for both the unwinding data [which is from the preceding paper (Roman & Kowalczykowski, 1989)] and the ATP hydrolysis data is below the apparent K_m values, maximum efficiency is not to be expected. The observed efficiency under these conditions does not change with temperature and is equal to approximately 5.0 ± 1.0.

An Arrhenius plot of the rate data yields an apparent energy of activation (E_a) for ATPase activity equal to 6.9 kcal (not shown). This value is approximately 70% of the value calculated for the helicase activity of recBCD enzyme (Roman & Kowalczykowski, 1989), further suggesting that the ATPase and helicase activities of recBCD enzyme have related, though not identical, kinetic properties.

Effect of Enzyme Concentration on RecBCD Enzyme dsDNA-Dependent ATPase Activity. RecBCD enzyme concentration was varied at 1.7 nM DNA molecules, a concentration of DNA approximately 10 times the apparent K_m value, to determine the stoichiometry of recBCD enzyme binding (not shown). The initial rate of ATP hydrolysis saturates at 35.7 ± 2.6 molecules of recBCD enzyme per dsDNA molecule. Thus, the stoichiometries of both the helicase (33 molecules of recBCD enzyme per dsDNA molecule; Roman & Kowalczykowski, 1989) and ATPase activities of recBCD enzyme are equal, within experimental error. For consistency, all the apparent k_{cat} values in both papers are corrected by a factor of 16.5 [derived from Roman and Kowalczykowski (1989)] to yield corrected k_{cat} values. The correction assumes a stoichiometry of one functional recBCD complex per dsDNA end. The sole purpose of this correction is to determine the true catalytic turnover number per functional enzyme (rather than total molar concentration) and makes no assumption as to the nature of the functional complex of recBCD enzyme.

A preparation of recBCD enzyme with a specific activity approximately 3-fold higher than that above was also used to determine the stoichiometry of recBCD binding. The initial rate of unwinding in this case showed saturation at 8.7 ± 3 molecules of recBCD enzyme per dsDNA molecule, 26% of that determined above. The rate observed at saturation in the presence of 1.7 nM DNA molecules is 2500 nM ATP/s, and the concentration of functional recBCD–dsDNA complex (i.e., dsDNA ends) is 3.4 nM; the resulting value for the turnover number is 735 μM ATP s^{-1} (μM functional recBCD complex)$^{-1}$. This is equal, within experimental error, to the apparent turnover number which has been corrected for the experimentally observed stoichiometry [740 ± 21 μM ATP s^{-1} (μM functional recBCD complex)$^{-1}$; Table II].

It is interesting to note that both the unwinding activity and the ATP hydrolysis activity both saturate at the same protein to DNA ratio, implying that only the molecules which are actively involved in unwinding dsDNA are hydrolyzing ATP.

Table IV: Kinetic Parameters, K_m and k_{cat}, for dsDNA in the ATPase Assay as a Function of Sodium Chloride Concentration[a]

row	[NaCl] (mM)	app K_m (nM DNA molecules)	app k_{cat} (s^{-1})	cor k_{cat}[b] (s^{-1})
1	0	0.1 ± 0.02	45 ± 1	740 ± 21
2	50	0.2 ± 0.1	50 ± 4	830 ± 64
3	100	0.2 ± 0.02	38 ± 2	630 ± 25
4	150	0.3 ± 0.03	48 ± 2	790 ± 31
5	200	0.2 ± 0.1	41 ± 7	680 ± 120

[a] All reactions were performed at 25 °C in standard buffer conditions containing 2.4 nM recBCD enzyme and enough SSB protein to saturate an equivalent amount of ssDNA 1.5-fold. [b] The "corrected k_{cat}" is the "apparent k_{cat}" corrected for the observed stoichiometry of recBCD enzyme binding to dsDNA, as for Table II.

Table V: Efficiency of ATP Utilization during DNA Unwinding

row	[NaCl] (mM)	efficiency[a] (molecules of ATP/base pair)
1	0	3.0 ± 0.3
2	50	2.4 ± 1.0
3	100	1.3 ± 0.4
4	150	1.9 ± 0.2
5	200	2.4 ± 0.5

[a] Determined as the ratio of the k_{cat} value for the ATPase activity relative to that for the helicase activity. The k_{cat} values for ATP hydrolysis are from Table IV, and the k_{cat} values for DNA unwinding are from Table II in Roman and Kowalczykowski (1989).

This result further substantiates the relationship established between the fast component of the dsDNA-dependent ATPase activity and the helicase activity.

Effect of Sodium Chloride on RecBCD Enzyme dsDNA-Dependent ATPase Activity. Sodium chloride has been shown to affect the maximum rate of dsDNA unwinding (Roman & Kowalczykowski, 1989). To determine the effect of sodium chloride on the ATPase activity, dsDNA concentration was varied at different salt concentrations, and apparent K_m and apparent k_{cat} values were determined (Table IV). The apparent K_m value increases slightly at the higher salt concentrations; a similar trend was also observed in the DNA helicase activity (Roman & Kowalczykowski, 1989).

The efficiency of ATP hydrolysis at various salt concentrations is shown in Table V. Little difference in efficiency is observed under conditions of maximum activity for both reactions, although there is a slight optimum at 100 mM sodium chloride. Thus, the ATPase and helicase activities of recBCD enzyme remain closely related even in increasing sodium chloride concentrations. If the apparent efficiency is calculated at low DNA concentrations (i.e., 0.14 nM DNA molecules) at various sodium chloride concentrations, this apparent efficiency changes with increasing salt concentration (not shown). At 0 mM sodium chloride, 4.5 ATP molecules are hydrolyzed per base pair unwound. At 100 and 200 mM sodium chloride, however, the apparent efficiencies become 5.5 and 17 ATP molecules hydrolyzed per base pair unwound, respectively. This variation in the observed ATP utilization is, again, to be expected due to the difference in apparent K_m values between the helicase and ATPase activities at these different sodium chloride concentrations.

Effect of Magnesium Acetate on RecBCD Enzyme dsDNA-Dependent ATPase Activity. Magnesium acetate was shown in the previous paper to have no effect on the initial rate of unwinding up to a concentration of 10 mM (Roman & Kowalczykowski, 1989). To determine whether the initial rate of ATPase activity exhibits the same behavior, magnesium acetate concentration was varied. As can be seen in Table III, rows 14–19, the initial rate of ATP hydrolysis increases with increasing magnesium acetate concentration. At 10 mM magnesium acetate, the initial rate is approximately one-third higher than that seen in 1 mM magnesium ion. The apparent efficiency of ATP hydrolysis as a function of magnesium acetate concentration is also shown in Table III. At 1 mM magnesium ion, the efficiency is 4.3, while at 10 mM magnesium ion, the apparent efficiency becomes 6 molecules of ATP hydrolyzed per base pair unwound. These experiments were performed at a low DNA concentration (0.14 nM) where the efficiency of ATP utilization became worse with increasing sodium chloride concentration.

Discussion

We have shown that recBCD enzyme has an ATPase activity which exhibits biphasic behavior when linear dsDNA is the substrate. The first phase of this reaction represents DNA unwinding since it correlates very well temporally to the helicase assay described in the preceding paper (Roman & Kowalczykowski, 1989). Comparison of the catalytic turnover values for this component of the ATP hydrolysis reaction with data from recBCD helicase activity yields an efficiency of ATP utilization of two to three ATP molecules hydrolyzed per base pair unwound. The second, slower component of the reaction results from the ssDNA-dependent ATP hydrolysis reaction following dsDNA unwinding. In the absence of SSB protein, this second phase is faster than in its presence, as is the rate of ssDNA-dependent ATPase activity, perhaps due to a competition between SSB and recBCD proteins for sites on the ssDNA. In the presence of SSB protein, recBCD-dependent ATPase activity of the slow phase is completely inhibited. The small amount of ATP hydrolysis observed in the slow phase in the presence of SSB protein is DNA independent and of an unknown origin.

The results obtained from the ATPase assay directly parallel those obtained from analysis of the recBCD helicase activity under the same conditions (Roman & Kowalczykowski, 1989). Supplementing an ATP hydrolysis reaction with additional recBCD enzyme after the fast kinetic component is completed does not result in a repeat of the biphasic behavior, indicating that the dsDNA present has been fully unwound with regard to recBCD enzyme function. If a further aliquot of dsDNA is added at the plateau of the ATP hydrolysis time course, an additional biphasic curve is produced, indicating that the second amount of dsDNA is also being unwound. If ssDNA is added to an ongoing ATPase assay containing dsDNA and SSB protein, the reaction proceeds as if the ssDNA was not present. If the ssDNA is present before the addition of the dsDNA, the reaction again proceeds as if the ssDNA is not present. The exact same results are obtained in the helicase assays (Roman & Kowalczykowski, 1989). These data further support the contention that the first phase of the ATPase activity is related to the unwinding of dsDNA.

The recBCD enzyme concentration dependence of ATP hydrolysis yielded a stoichiometry of 35.7 recBCD enzyme molecules per DNA molecule. This is similar to the stoichiometry obtained from DNA helicase assays, and, as discussed previously (Roman & Kowalczykowski, 1989), this stoichiometry is greater than expected probably due to the presence of inactive recBCD enzyme molecules in our preparation. Whatever the molecular basis for this observed stoichiometry, both the helicase and ATPase activities of recBCD enzyme saturate at the same value. This implies that only the molecules which are actively hydrolyzing ATP are involved in the unwinding of dsDNA. That is, there are no recBCD molecules that are nonspecifically bound to the in-

ternal base pairs of the dsDNA hydrolyzing ATP. This is consistent with the observations that circular RF DNA does not compete with duplex ends either in an assay of recBCD enzyme ATPase activity (Karu et al., 1973) or in its helicase activity (Roman & Kowalczykowski, 1989).

We have determined apparent K_m and apparent k_{cat} values for both ATP and dsDNA molecules in the ATPase assay. The ATP turnover number is 45 μM ATP s^{-1} (μM recBCD enzyme)$^{-1}$ [or 740 μM ATP s^{-1} (μM functional recBCD complex)$^{-1}$ if corrected for the observed stoichiometry, as in Roman and Kowalczykowski (1989)]. An efficiency of ATP hydrolysis during unwinding of dsDNA by recBCD enzyme can be calculated from the maximum rates (i.e., apparent k_{cat} values) of ATPase and helicase activities. Under these conditions, the efficiency is two to three ATP molecules hydrolyzed per base pair unwound by recBCD enzyme. The high efficiency of ATP hydrolysis during unwinding and the very close parallel between helicase and ATPase activities indicate that these two activities are closely related. At best, under optimal conditions, the free energy derived from ATP hydrolysis (−11.3 kcal/mol as calculated at 25 °C in the presence of 3 mM MgATP, 0.1 mM MgADP, 1 mM MgP_i, and 1 mM magnesium ion) could only "open" two to four base pairs of dsDNA per ATP molecule hydrolyzed (assuming that the standard-state free energy of denaturation is approximately 2.5 and 5.0 kcal/mol for A-T and G-C base pairs, respectively). The fact that recBCD enzyme hydrolyzes only 3.0 ATP molecules per base pair unwound means that this enzyme is relatively energy efficient.

Although the helicase and ATPase activities of recBCD enzyme appear to be very closely related, with the energy derived from hydrolysis most likely being used to unwind the dsDNA, under certain, suboptimal conditions these activities become separable. This implies that these activities are not directly coupled in the strictest mechanistic sense; ATP hydrolysis does not occur only upon DNA unwinding and *vice versa*. This is demonstrated by the observation that the number of ATP molecules hydrolyzed per base pair unwound rises at low DNA concentration and at higher sodium chloride or magnesium acetate concentration (Table III). In the presence of 100 or 200 mM sodium chloride, these apparent efficiencies become 6 and 17, respectively. However, at high DNA concentrations (i.e., under conditions of maximum rate of unwinding), the efficiency of ATP hydrolysis is only slightly affected by salt concentration. This result is to be expected since the apparent K_m values for dsDNA differ significantly between the ATPase and helicase assays while the maximum rates of unwinding and ATP hydrolysis are less affected by the salt concentration.

The observation that recBCD enzyme has ssDNA-dependent ATPase activity indicates that unwinding of the DNA need not occur while ATP is hydrolyzed. Lack of direct coupling between the ATPase activity of recBCD enzyme and its helicase or nuclease activities was demonstrated most clearly by Karu and Linn (1972), who showed that the ATPase activity of recBCD enzyme is not affected by the introduction of cross-links into the dsDNA molecule whereas nuclease activity and, presumably, helicase activity are inhibited. The fact that the ssDNA endonuclease activity is not dependent on the presence of ATP (Goldmark & Linn, 1970) shows that ATP hydrolysis is not necessarily required for nuclease activity. No conditions have been found, however, under which unwinding occurs in the absence of ATP hydrolysis.

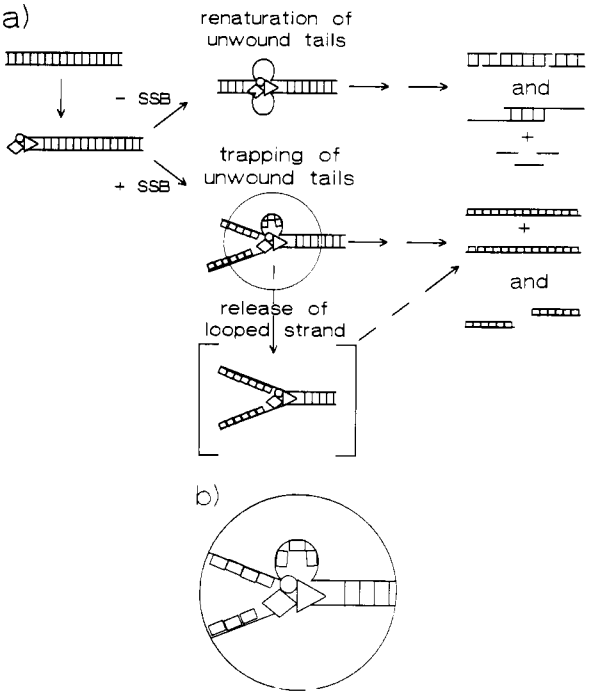

FIGURE 5: Model of recBCD enzyme helicase activity. Triangles, diamonds, and circles represent the recB, recC, and recD protein subunits, respectively. Squares represent SSB protein. Specific details are discussed in the text.

The apparent K_m values for ATP and dsDNA in the ATPase assay are 85 ± 15 μM ATP and 0.13 ± 0.02 nM dsDNA molecules, respectively. These apparent K_m values are well below the concentrations expected in vivo. The in vivo concentration of ATP is approximately 3 mM, and the molar concentration of one dsDNA end in an *E. coli* cell is calculated to be 0.9 nM (Roman & Kowalczykowski, 1989).

An integrated model describing the helicase and ATPase activities of recBCD enzyme is shown in Figure 5. Figure 5 depicts the unwinding of dsDNA as derived from electron micrographs (Taylor & Smith, 1980; Telander-Muskavitch & Linn, 1980). RecBCD enzyme first binds to an end of the dsDNA molecule (Taylor & Smith, 1985) and begins to unwind it. In the absence of SSB protein (top pathway), the unwound strands quickly reanneal, and a "twin loop" structure is formed. RecBCD enzyme continues translocating through the molecule, introducing random nicks along its length. In the presence of a sufficient excess of SSB protein (or gene 32 protein) (lower pathway), the ssDNA produced by recBCD enzyme is trapped by SSB protein and prevented from reannealing, thus forming a "loop-tailed" structure. "Forked" DNA structures are also seen in the electron microscope and are probably decay products of the "loop-tail" structure (Telander-Muskavitch & Linn, 1982) in which recBCD enzyme has dissociated from the molecule or "dropped" one strand, as discussed below. Normally, recBCD enzyme continues unwinding a DNA molecule the size of M13 until fully ssDNA products are formed. Thus, the products of recBCD enzyme helicase activity are very well characterized, but the actual mechanism of unwinding and the roles of the recB, recC, and recD subunits are still unclear. Recent findings, together with our own, provide some insight into these mechanistic questions.

Intuitively, since loops are formed in the dsDNA, recBCD enzyme must contact the dsDNA at a minimum of two different sites. The contact sites can either be on the same strand of dsDNA or be on opposite strands. In either case, recBCD enzyme would unwind dsDNA ahead of itself. These strands are then "rewound" after recBCD enzyme has passed through.

We would like to suggest a mechanism by which recBCD enzyme unwinds dsDNA via contacts with both strands of dsDNA (Figure 5b).

Several observations support our proposal that recBCD enzyme contacts both strands of the dsDNA rather than a single strand. First, recBCD enzyme requires dsDNA substrates either with a blunt end or with short (<25 nucleotides) ssDNA tails (Taylor & Smith, 1985). It cannot initiate unwinding when longer ssDNA tails are present, suggesting that recBCD enzyme must contact both strands of dsDNA and cannot translocate along a ssDNA tail to gain entry into a dsDNA region. Second, the polarity of the short ssDNA tails is unimportant (Taylor & Smith, 1985), again implying that recBCD enzyme does not simply travel directionally along a single ssDNA strand. A dsDNA molecule has no intrinsic polarity, again suggesting that recBCD enzyme binds to and moves along *both* strands of dsDNA (see below). Finally, we show that ssDNA is 10-fold poorer at stimulating the ATPase activity of recBCD enzyme; this implies that full (ATPase) activity of the enzyme is effected only when it can contact both strands of the dsDNA. Since ATP hydrolysis is presumably required for translocation through the dsDNA, our results argue that the recBCD enzyme translocates poorly, if at all, along ssDNA. This is consistent with the implications of Taylor and Smith's work (1985) discussed above.

Which subunits actually contact the DNA strands during translocation? The following experimental data suggest that it is the recB and recD subunits. Finch et al. (1986a) have shown that the recB subunit can bind to ssDNA but recC subunit cannot. The recD subunit was not tested but is another likely candidate, since it possesses an ATP binding site (see below). On the basis of the studies of the Smith laboratory, mutations in the recC subunit (recC* and TexA mutants) result in a recBCD enzyme proficient in nuclease activity but lacking in χ cutting activity (Schultz et al., 1983), suggesting that the recC subunit is the site of χ recognition. Finch et al. (1986a–c) have sequenced the recB, recC, and recD subunits and have found nucleotide binding sites [described by Walker et al. (1982)] in the recB and recD subunits, but not in the recC subunit. The recB subunit alone can catalyze the hydrolysis of ATP (Hickson et al., 1985), but ATP hydrolysis has not been detected with either the recC (Hickson et al., 1984) or the recD subunits alone (Lieberman & Oishi, 1974). However, Julin and Lehman (1987) have shown that 8-azido-ATP will label two subunits of intact recBCD enzyme (recB and recD proteins), suggesting that the recD subunit must be complexed with the recB and recC subunits to be active.

The dissociation constants for 8-azido-ATP binding differ for each of the subunits and are approximately 30 and 120 μM for recD and recB subunits, respectively (Julin & Lehman, 1987). These values are similar to the apparent K_m values we determined for ATPase and helicase activities, 85 ± 15 and 130 ± 30 μM, respectively (Roman & Kowalczykowski, 1989) (although the large experimental error in these values precludes an unequivocal statement that the apparent K_m values for ATP in the helicase and ATPase activities are different). The difference in apparent K_m values for ATP and for dsDNA derived from the ATPase and helicase assays (Table II) might suggest that dsDNA-dependent ATPase activity is governed by the kinetic activation of the subunit with the higher ATP affinity (recD) but that activation of both subunits (recB and recD) is required for unwinding activity.

In the simplest model for recBCD enzyme translocation, the hydrolysis of one ATP molecule is catalyzed by each of the recB and recD subunits, which are bound to opposite strands of the DNA molecule, thus resulting in the "walking" of the recBCD enzyme down the DNA molecule. The "looped" structures can be explained if one of the subunits hydrolyzes ATP at a slower rate and thus translocates down its strand of DNA more slowly. Thus, if one subunit (shown as recB in Figure 5b) is translocating along the strand to which it is bound at 300 bases/s [as observed by Taylor and Smith (1980)] while the other subunit (shown as recD in Figure 5b) is only translocating along the strand to which it is bound at 200 bases/s, the loop would enlarge at a rate of 100 bases/s [as observed by Taylor and Smith (1980)]. If SSB protein is present, a "loop-tailed" structure would be formed. In the absence of SSB protein, the strands would reanneal behind the loop and cause the unlooped strand to also bow out, thus forming "twin-loops". A "forked" structure would be formed in the presence of SSB protein if the recD subunit released the strand to which it was bound, thus releasing the constraint forming the loop.

Our observation that approximately two (to three) ATP molecules are hydrolyzed per base pair unwound is also suggestive. If each subunit must hydrolyze 1 ATP molecule to translocate 1 nucleotide, the recB subunit would hydrolyze 1 ATP molecule for each base pair unwound while the recD subunit, which translocates only two-thirds as fast as the recB subunit, would hydrolyze only 0.66 ATP molecule for each base pair unwound. Thus, the total ATP requirement would be 1.66 ATP molecules hydrolyzed per base pair unwound, which is nearly (perhaps fortuitously) the value we observe under the most efficient conditions (Table V).

An integrated picture of the recBCD enzyme can be drawn as follows. The enzyme is a helicase which efficiently uses the energy derived from the hydrolysis of ATP molecules to unwind dsDNA. For helicase activity, the hydrolysis of approximately two ATP molecules is a necessary, though not sufficient, condition for the unwinding of one base pair of dsDNA. Conversely, however, DNA unwinding is not essential for ATPase activity; perhaps recBCD enzyme can "sit" in place and hydrolyze ATP without advancing through the dsDNA. RecBCD enzyme also has a random nuclease activity (with a marked preference for χ sequences; Taylor et al., 1985) whereby it nicks one strand of the dsDNA while it is unwinding. This nicking activity appears to be constant over time (unless inhibited by calcium or SSB protein) rather than dependent on the number of nucleotides unwound. The nuclease activity is affected by a number of factors (i.e., calcium ion, SSB protein, or ATP concentrations) independently of the helicase and ATPase activities. Since the rate of DNA unwinding is increased to a greater extent than is the nuclease activity by increasing ATP concentration, increasing ATP concentration results in the formation of longer product molecules (Mackay & Linn, 1976). More work is necessary to fully understand the actual mechanism by which ATP hydrolysis and the unwinding of dsDNA by recBCD enzyme are related, and this model will be a useful framework for future studies.

Finally, we have proposed conditions for the definition of a helicase unit for the activity of recBCD enzyme (Roman & Kowalczykowski, 1989) and now wish to define an ATPase unit, which is much easier to measure. We suggest that 1 unit of recBCD enzyme be equal to the amount required to hydrolyze 1.0 nmol of ATP in 1 min at 25 °C, in standard buffer containing 1 mM magnesium acetate, 1 mM ATP, and 0.6 nM dsDNA molecules. Additionally, since the apparent K_m value for DNA in the ATPase assay is much lower than 0.6 nM, the measured rate would be approximately equal to the

apparent k_{cat}. Thus, the efficiency of ATP hydrolysis during unwinding can easily be calculated from the data used to define the helicase and ATPase units.

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Registry No. ATPase, 9000-83-3; ATP, 56-65-5; magnesium, 7439-95-4.

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Solution Structure of the Glycosylphosphatidylinositol Membrane Anchor Glycan of *Trypanosoma brucei* Variant Surface Glycoprotein†

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ABSTRACT: The average solution conformation of the glycosylphosphatidylinositol (GPI) membrane anchor of *Trypanosoma brucei* variant surface glycoprotein (VSG) has been determined by using a combination of two-dimensional ^1H–^1H NMR methods together with molecular orbital calculations and restrained molecular dynamics simulations. This allows the generation of a model to describe the orientation of the glycan with respect to the membrane. This shows that the glycan exists in an extended configuration along the plane of the membrane and spans an area of 600 Å^2, which is similar to the cross-sectional area of a monomeric N-terminal VSG domain. Taken together, these observations suggest a possible space-filling role for the GPI anchor that may maintain the integrity of the VSG coat. The potential importance of the GPI glycan as a chemotherapeutic target is discussed in light of these observations.

The parasitic protozoan *Trypanosoma brucei* undergoes a complex life cycle between an insect (tsetse fly) vector and its mammalian hosts. *T. brucei* is the causative agent of ngana in cattle and is closely related to *Trypanosoma rhodesiense* and *Trypanosoma gambiense*, which cause human sleeping sickness. The parasite lives in the blood and lymph of the mammalian host where it is protected from lytic serum components by a dense monolayer of variant surface glycoprotein (VSG)[1] that forms a continuous macromolecular diffusion

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[1] Abbreviations: GPI, glycosylphosphatidylinositol; VSG, variant surface glycoprotein; COSY, homonuclear correlated spectroscopy; NOESY, homonuclear nuclear Overhauser effect spectroscopy; NMR, nuclear magnetic resonance; MO, molecular orbital; MM, molecular mechanics; MD, molecular dynamics.

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