Efficiency of ATP Hydrolysis and DNA Unwinding by the RecBC Enzyme from *Escherichia coli*†

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ABSTRACT: We have measured the rates and efficiencies of DNA unwinding (the number of ATP molecules hydrolyzed per DNA base pair unwound) catalyzed by the RecBC, RecBCD-K177Q (a site-directed mutant in the putative ATP-binding site in the RecD subunit), and RecBCD enzymes from *Escherichia coli*. The DNA unwinding rate was measured with a coupled assay in which unwound DNA is degraded by the combined action of the RecJ enzyme and exonuclease I. The rates of DNA unwinding by the RecBC and RecBCD-K177Q enzymes are reduced by about 4-fold compared to the case of the RecBCD enzyme. The efficiency of ATP hydrolysis was determined in two ways. First, it was calculated from the ratio of the ATP hydrolysis rate to the rate of DNA unwinding. In the second method, ATP hydrolysis was measured under conditions where all of the DNA substrate becomes completely unwound. The efficiency is the ratio of the total amount of ATP hydrolyzed to the amount of DNA substrate present in the reaction. The average efficiencies measured kinetically and by the complete unwinding experiment are as follows: 2.30 and 1.74 ATP/base pair (RecBCD enzyme); 1.44 and 1.28 (RecBC); and 1.20 and 1.07 (RecBCD-K177Q). The RecBC and RecBCD-K177Q enzymes are therefore able to couple ATP hydrolysis to DNA unwinding at least as efficiently as the RecBCD holoenzyme. The lower ATP per base pair ratios found for RecBC and RecBCD-K177Q indicate that the RecD subunit hydrolyzes ATP during DNA unwinding by the RecBCD enzyme.

The RecBCD enzyme from *Escherichia coli* consists of the protein products of the *recB*, *recC*, and *recD* genes (reviewed in Taylor (1988) and Smith (1990)). The enzyme has several catalytic activities, including DNA-dependent ATPase, ATP-dependent nuclease on single- or double-stranded DNA, and ATP-dependent helicase. Cells with null mutations in either the *recB* or *recC* gene are deficient in homologous recombination, are sensitive to ultraviolet light, X-rays, and chemical DNA-damaging agents, and have low viability (Smith, 1990). These cells also lack the enzymatic activities of the RecBCD enzyme (Smith, 1990). Null mutants in the *recD* gene (Chaudhury & Smith, 1984) are rather different from *recB* and *recC* mutants, as they are recombination proficient, although the recombination is no longer stimulated by the recombination hot spot Chi. These mutants are, however, UV sensitive and have low viability (Chaudhury & Smith, 1984). Crude extracts of the *recD* mutant cells lack the ATP-dependent nuclease activity on linear double-stranded DNA which is characteristic of the RecBCD enzyme (Chaudhury & Smith, 1984). Their recombination proficiency suggested that the RecBC enzyme, lacking the RecD subunit, retained some activity of the RecBCD enzyme crucial to recombination. The nature of this activity was unclear, since the extracts from the *recD* mutant cells apparently also lacked the DNA unwinding activity of the RecBCD enzyme (Amundsen et al., 1990).

We are interested in understanding the functions of the individual subunits, particularly RecD, and their interactions with ATP, in the catalytic activities of the RecBCD enzyme. Our efforts have focused on examining the catalytic activities of site-directed mutants and of the purified subunits. The RecBCD enzyme couples the energy from ATP hydrolysis to rapid and processive unwinding and degradation of double-stranded DNA (Muskavitch & Linn, 1982; Roman & Kowalczykowski, 1989a; Roman et al., 1992; Korangy & Julin, 1992c). Both the RecB and RecD subunits bind ATP (Julin & Lehman, 1987) and have amino acid sequence similarity to several other DNA helicases (Gorbalenya et al., 1988; Hodgman, 1988). The RecB protein by itself is a DNA-dependent ATPase (Hickson et al., 1985; Boehmer & Emmerson, 1992; Masterson et al., 1992) and a weak DNA helicase (Boehmer & Emmerson, 1992). The RecBC enzyme also has DNA helicase activity *in vitro* (Palas & Kushner, 1990; Masterson et al., 1992; Korangy & Julin, 1993) and *in vivo* (Rinken et al., 1992), as does an enzyme with a mutation in the consensus ATP-binding sequence of the RecD subunit (RecBCD-K177Q enzyme; Korangy & Julin, 1992b). These observations show that there is no absolute requirement for RecD in DNA unwinding by the RecBCD enzyme. However, both the RecBC and RecBCD-K177Q enzymes are slower and less processive than RecBCD (Korangy & Julin, 1992c; Korangy & Julin, 1993), suggesting that RecD does have some role in the unwinding reaction. An enzyme with a site-directed mutation in the consensus ATP-binding sequence of the RecB subunit (RecB-K29Q-CD enzyme) is inactive on blunt-ended double-stranded DNA (Hsieh & Julin, 1992), consistent with a requirement for ATP hydrolysis by RecB for DNA unwinding. This mutant retains single-stranded DNA-dependent ATPase activity, suggesting that the RecD subunit is able to catalyze ATP hydrolysis. The RecB-K29Q-CD mutant enzyme is also an ATP-dependent nuclease on single-stranded DNA.

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We report here measurements of the rate of DNA unwinding and the efficiency of coupling between ATP hydrolysis and DNA unwinding by the RecBC and RecBCD-K177Q enzymes, for comparison to the RecBCD enzyme. We used two experimental approaches to measure the efficiency: (1) parallel measurement of the initial rates of ATP hydrolysis and DNA unwinding and (2) measurement of the total amount of ATP hydrolyzed during complete unwinding of all the DNA substrate presented to the enzyme. We find that the RecBCD enzyme hydrolyzes about two ATP molecules for every base pair unwound (2.30 and 1.74 ATP/bp[1] from the two experimental approaches), in good agreement with previous work (Roman & Kowalczykowski, 1989b). The RecBC and RecBCD-K177Q enzymes hydrolyze fewer ATPs per DNA base pair unwound (1.44 and 1.28, and 1.20 and 1.07 ATP/bp, respectively). These results are consistent with a mechanism in which the RecD subunit hydrolyzes ATP during DNA unwinding by the RecBCD enzyme, but this ATP hydrolysis is not required for the unwinding reaction itself. We suggest that it may enable the enzyme to translocate along the unwound DNA and/or have a direct role in the nuclease reaction.

EXPERIMENTAL PROCEDURES

Materials. ATP was purchased as a 100 mM solution from Pharmacia Corp. [methyl-^{3}H]Thymidine was from Amersham, and [γ-^{32}P]ATP was from New England Nuclear. ExoI and S1 nuclease were purchased from U.S. Biochemicals Corp., and restriction endonucleases were from U.S. Biochemicals Corp. or Promega. The RecJ exonuclease (Lovett & Kolodner, 1989) was a generous gift from Dr. Richard Kolodner, Harvard University. The SSB protein from *E. coli* was purified as described (Lohman et al., 1986), with further purification by chromatography on hydroxylapatite to remove contaminating nuclease and ATPase activities.

Enzymes. The RecBCD and RecBCD-K177Q enzymes were prepared as described (Korangy & Julin, 1992a). The RecB and RecC proteins were purified as described by Masterson et al. (1992). The concentration of each enzyme was determined from the absorbance at 280 nm using absorption coefficients of 400 000 (RecBCD and RecBCD-K177Q (Roman & Kowalczykowski, 1989a)), 170 000 (RecB), and 200 000 M^{-1} cm^{-1} (RecC). The latter were calculated from the known content of aromatic amino acids in these proteins (Finch et al., 1986a,b). The RecB and RecC proteins were mixed in a ratio of 1.7–2 RecC/1 RecB and incubated on ice for at least 30 min in the buffer used by Masterson et al. (1992), to form the RecBC enzyme. The RecBC enzyme concentration was taken to be equal to that of the RecB subunit, since its concentration was limiting.

We estimated previously that at least 33% of the RecBC enzyme produced by incubating the individual subunits is active enzyme (Korangy & Julin, 1993). We checked this again by measuring the initial rate of ATP hydrolysis at various ratios of RecBC enzyme to the DNA ends. The assumption is that the rate is maximal at one active enzyme per end, when the DNA ends concentration is saturating relative to the apparent K_m for the DNA (Roman & Kowalczykowski, 1989a). The rate of ATP hydrolysis was maximal with about two RecBC enzymes per end and did not increase significantly with up to six RecBC per end, with 12.8 or 19.2 nM DNA ends and 10 μM ATP. This indicates that the RecBC enzyme is at least 50% active, in good agreement with the previous estimate. Similar measurements of the nuclease activity of the RecBCD and RecBCD-K177Q enzymes indicate that these enzyme preparations consist of essentially 100% active enzyme (Korangy & Julin, 1992b). The RecBC concentrations and rates for this enzyme reported herein (Table 1) have been corrected for this apparently inactive enzyme by dividing the RecBC concentrations (determined spectrophotometrically) by 2. The values for the efficiencies are unlikely to be affected by uncertainties in the precise percentage of active enzyme in our preparations. We note also that the quaternary structure of the active form of RecBC(D) on the DNA is not yet known.

DNA Substrates. The plasmid DNA substrates pPvSm19 (6.25 kb), pTZ19R (2.86 kb), and pDJ01 (21.4 kb) were prepared as described (Korangy & Julin, 1992b,c). The circular DNA was linearized by treatment with restriction enzymes *Sac*I, *Kpn*I, or *Bgl*I. These enzymes produce 3′-terminal single-stranded overhangs of four nucleotides (*Sac*I and *Kpn*I) or three nucleotides (*Bgl*I). The RecBC enzyme has preference for double-stranded DNA substrates with 3′-overhangs, as compared to blunt ends or four-nucleotide 5′-overhangs (Korangy & Julin, 1993). *Bgl*I cleaves pTZ19R to produce two fragments, of 1595 and 1268 bp, which were added together to reaction mixtures. Denatured substrates were prepared by immersing linearized DNA in a boiling-water bath for 5 min and then immediatley transferring the tube to ice water.

The concentrations of double-stranded DNA stock solutions were determined from the absorbance at 260 nm using ϵ_{260} = 6490 (M nucleotides)$^{-1}$ cm^{-1}. The concentrations were also determined by fluorescence in the presence of Hoechst 33258 dye using a TKO 100 fluorometer (Hoefer Scientific Instruments). The standard for the fluorescence measurements was plasmid DNA (circular or linearized) whose concentration had been determined by absorbance measurements. The specific radioactivity of the [^{3}H]DNA was determined by scintillation counting of small aliquots in mixtures containing the same concentration of trichloroacetic acid as would be present in quenched nuclease reaction aliquots.

ATP Hydrolysis. The standard reaction conditions were 50 mM Tris·HCl, pH 7.5, 10 mM $MgCl_2$, and 0.67 mM DTT, with SSB, [γ-^{32}P]ATP, DNA, and RecBC(D) enzyme at concentrations specific for each experiment. All reactions were done at 37 °C. The concentrations of ATP stock solutions (before [γ-^{32}P]ATP was added) were determined from the absorbance at 259 nm using ϵ_{259} = 1.54 × 10^4 M^{-1} cm^{-1} (Maniatis et al., 1982). ATP hydrolysis was measured using the polyethyleneimine thin-layer chromatography assay used previously (Korangy & Julin, 1992b). ATP hydrolysis rates were determined from the slope of linear least squares fits of the time courses, using data obtained before about 20% of the limiting substrate (usually the DNA) was consumed.

DNA Unwinding Measurements. For RecJ/exoI coupled reactions, the RecBC(D) enzyme was first mixed with the DNA and other components without ATP, in the standard reaction conditions at 37 °C. RecJ exonuclease (0.9 units/μL) and exoI (0.04 units/μL) were then added, followed immediately (<10 s) by ATP to start the reaction. Aliquots removed from the reaction mixtures were treated with trichloroacetic acid as described (Korangy & Julin, 1992b) to determine the acid-soluble DNA present. The amount of acid-soluble tritium was converted to the molar concentration of soluble DNA nucleotides using the known specific radioactivity of the DNA. The initial reaction rate was calculated as above.

[1] Abbreviations: bp, base pairs; DTT, dithiothreitol; exoI, exonuclease I from *E. coli*; kb, kilobase pairs; nt, nucleotide; RecBC(D), the RecBC, RecBCD, or RecBCD-K177Q enzyme; RecJ, the RecJ exonuclease from *E. coli*; SDS, sodium dodecyl sulfate; SSB, single-stranded DNA binding protein from *E. coli*.

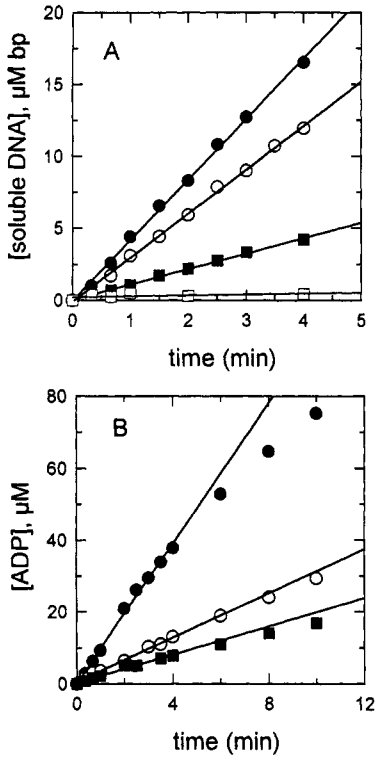


FIGURE 1: DNA unwinding and ATP hydrolysis by the RecBCD, RecBCD-K177Q, and RecBC enzymes. Reaction mixtures (standard conditions) contained pPvSm19 [^{3}H]DNA linearized with *Sac*I (50 μM bp), 200 μM ATP ([γ-^{32}P]-labeled or unlabeled), and 8 μM SSB protein. The reactions were initiated by adding a mixture of ATP, RecJ (0.94 units/μL), and exoI (0.046 units/μL). (●) RecBCD enzyme (0.081 nM); (○) RecBCD-K177Q enzyme (0.25 nM); (■) RecBC enzyme (0.088 nM); (□) no added RecBC(D) enzyme. A. DNA unwinding measured by the RecJ/exoI coupled assay (see Experimental Procedures). B. ATP hydrolysis.

Only a very small amount (less than 1%) of the DNA substrate was solubilized by RecJ and exoI alone, in the absence of RecBC(D), and this background was ignored.

S1 nuclease coupled reactions were done as described (Korangy & Julin, 1992b). Samples removed from RecBC(D) enzyme reaction mixtures were treated with 0.03 units/μL of S1 nuclease in the presence of 0.3% SDS at 37 °C for 30 min. The acid-soluble [^{3}H]DNA fragments were then determined as above. The background of DNA solubilized by S1 alone was subtracted from that produced by RecBC(D).

For agarose gels, aliquots from the reaction mixtures were quenched with 50 mM EDTA and 0.3% SDS and loaded on a 0.7% agarose gel. The gels were treated with EN^{3}HANCE and visualized by autofluorography as described (Korangy & Julin, 1992c).

RESULTS

DNA Unwinding Measured by the RecJ/exoI Coupled Assay. Figure 1A shows time courses of DNA unwinding catalyzed by the RecBC(D) enzymes, measured by the RecJ/exoI coupled assay. The initial rates of unwinding, divided by the enzyme concentration used in the particular measurement (μM ADP min^{-1} μM enzyme^{-1}), are summarized in Table 1. The values found for the RecBCD enzyme with pPvSm19 DNA are very close to the k_{cat} for the helicase activity at 37 °C measured previously by a different assay method (56 000 bp/min; Roman & Kowalczykowski, 1989a). The rates for the RecBC and RecBCD-K177Q enzymes at the highest DNA concentration we used here (50 μM bp) are reduced by about 4-fold for both the RecBC and RecBCD-K177Q enzymes compared to the RecBCD enzyme.

The nuclease activities of the RecBCD and RecBCD-K177Q enzymes produce a substantial amount of acid-soluble DNA, in addition to that produced by the coupling enzymes, under these reaction conditions (Korangy & Julin, 1992b). The nuclease activity of the RecBCD enzyme on double-stranded DNA requires ATP hydrolysis, translocation of the enzyme along the DNA, and concomitant DNA unwinding (Muskavitch & Linn, 1982). We therefore consider DNA unwinding by the RecBCD and RecBCD-K177Q enzymes in these experiments to be the total amount of DNA which becomes acid-soluble, due to either the ATP-dependent nuclease activity of RecBCD or RecBCD-K177Q or single-stranded DNA which is degraded by the coupling enzymes. The RecBC enzyme has little if any nuclease activity under these reaction conditions (Palas & Kushner, 1990; Masterson et al., 1992; Korangy & Julin, 1993), and so DNA unwinding alone is being measured with this enzyme.

In the coupled unwinding assay, unwound DNA produced by the RecBC(D) enzyme is prevented from reannealing by the SSB protein and made acid-soluble by the RecJ exonuclease and exoI. These enzymes degrade single-stranded DNA with opposite polarity (5′ to 3′ for RecJ, 3′ to 5′ for exoI (Kornberg & Baker, 1991)). Both enzymes are able to degrade single-stranded DNA coated with the SSB protein (data not shown). Several control experiments were done to validate the RecJ/exoI coupled unwinding assay. The results of one of these experiments are shown in Table 2. No significant change in the unwinding rates was seen if the concentration of RecJ/exoI or SSB protien was doubled, while the rate did increase when the RecBCD enzyme concentration was increased. The rate of unwinding by RecBC was at least 10-fold lower if SSB was omitted from the reaction mixture (data not shown). The unwound DNA strands presumably reanneal in the absence of SSB and become insensitive to RecJ/exoI.

In some experments, unwound DNA trapped by the SSB protein was degraded by S1 nuclease after the SSB was removed from the DNA with SDS (Abdel-Monem et al., 1977, Duguet et al, 1978). The unwinding rates obtained with the S1 nuclease assay (Table 1) are in good agreement with those from the RecJ/exoI coupled assay.

Initial Rates of ATP Hydrolysis. The initial rates of ATP hydrolysis catalyzed by each enzyme were measured in parallel with the unwinding measurements and under identical reaction conditions. Sample time courses are shown in Figure 1B, and the rates are summarized in Table 1.

The reductions in the ATP hydrolysis rates for RecBC (6-fold reduction compared to RecBCD) and RecBCD-K177Q enzyme (about 7-fold) are similar to those we measured before (Korangy & Julin, 1993). However, the ATP hydrolysis rates in Table 1 are somewhat faster than expected from the k_{cat} values we measured previously for these enzymes under similar reaction conditions but without SSB (Korangy & Julin, 1992b; Korangy & Julin, 1993[2]). This is largely due to stimulation of the ATPase reaction by SSB. The initial rate of double-stranded DNA-dependent ATP hydrolysis catalyzed by the RecBCD enzyme was 1.4 (±0.08)-fold greater with SSB protein present (8–16 μM), compared to reactions with no SSB, with 50 μM DNA bp (*Sac*I-cut pPvSm19), and 0.08 nM enzyme. Stimulation of about this magnitude was reported

[2] Note that the ATP hydrolysis rates for RecBC reported in Korangy and Julin (1993) were uncorrected.

Table 1: ATP Hydrolysis and DNA Unwinding Rates and Efficiency[a]

enzyme	DNA	[DNA] (μM bp)	ATP hydrolysis (±s.e.)[b] (min^{-1})	DNA unwinding (±s.e.)[b] (bp/min)	efficiency[c] (ATP/bp)	N[d]
RecBCD	pPvSm19/*Sac*I	50	103 000(4500)	49 000(5000)	2.16(0.39)	2
	(6250 bp)			47 000[e]		(1)
	pTZ19R/*Bgl*I	30	76 000(5000)	28 000(2250)	2.56(0.27)	6
	(1595,1268 bp)	50	81 000(2000)	44 600(1900)	1.83(0.09)	3
					overall average: 2.30(0.18)	11
RecBC	pPvSm19/*Sac*I	20	7300(1900)	4800(400)	1.50(0.24)	2
		50	16 000(1600)	11 600(700)	1.31(0.13)	7
				12 000(1700)[e]		(7)
	pTZ19R/*Bgl*I	10	7600(600)	5600(200)	1.36(0.13)	4
		30	11 600(800)	7800(500)	1.54(0.13)	12
		50	18 300(2600)	11 000(100)	1.32	1
		60	15 700(1100)			
					overall average: 1.44 (0.07)	26
RecBCD-K177Q	pPvSm19/*Sac*I	50	14 000(1100)	13 000(800)	1.04(0.16)	3
				12 500(2000)[e]		(2)
	pTZ19R/*Bgl*I	30	4600(600)	4200(700)	1.25(0.19)	3
		50	10 900(500)	8400(700)	1.33(0.17)	3
					overall average: 1.20(0.10)	9

[a] Reaction mixtures (standard conditions) contained 200 μM ATP, 8–24 μM SSB protein, 0.03 or 0.08 nM RecBCD enzyme, 0.08–0.24 nM RecBC enzyme, or 0.24–0.32 nM RecBCD-K177Q enzyme. [b] Initial reaction rate (μM ADP/min or μM bp/min) divided by the enzyme concentration (μM). Standard errors were calculated by s.e. = (standard deviation)/($N^{1/2}$). [c] Calculated from (ATP hydrolysis rate)/(DNA unwinding rate). The unwinding rates were those determined in the RecJ/exoI assay. [d] Number of determinations of the efficiency from side-by-side measurements of ATP hydrolysis and DNA unwinding. The rates of ATP hydrolysis and DNA unwinding are the averages of a greater number of determinations (2–20). [e] DNA unwinding measured using S1 nuclease.

Table 2: Effect of Variation in the Coupling Components on Unwinding Rates Measured in the RecJ/exoI Assay

reaction mixture	unwinding rate (μM bp/min)	relative rate
complete[a]	2.77	1.00
+2× RecJ/exoI	2.63	0.95
+2× SSB	2.34	0.84
+2× RecBCD	5.06	1.83

[a] Complete reaction mixtures (standard conditions) contained 200 μM ATP, 30 μM (bp) pTZ19R [^{3}H]DNA cleaved with *Bgl*I, 8 μM SSB (1×), 0.08 nM RecBCD enzyme, 0.85 units/μL RecJ (1×), and 0.04 units/μL exoI (1×). 2× indicates that the concentration of that component was doubled. The rates are averages of duplicate determinations.

in one previous study with the RecBCD enzyme (MacKay & Linn, 1976) but not in another (Roman & Kowalczykowski, 1989b). RecBC was stimulated by 2.32(±0.15)-fold under the same conditions, with 0.12 nM enzyme. An equivalent amount of SSB storage buffer had no effect on the ATP hydrolysis rate, and the SSB alone has no ATPase activity (data not shown). RecJ and exoI (in the presence of SSB) did not stimulate the rate of ATP hydrolysis catalyzed by the RecBC(D) enzymes. Single-stranded DNA-dependent ATP hydrolysis catalyzed by RecBC(D) was strongly inhibited by the SSB protein (data not shown), as observed before (McKay & Linn, 1976; Roman & Kowalczykowski, 1989b). The origin of the stimulation by SSB is unknown, but it may arise from the SSB preventing RecBC(D) from binding to single-stranded DNA present during DNA unwinding. In support of this possibility, we found no effect of SSB on the rate of ATP hydrolysis at low ATP concentrations (2 μM) catalyzed by either RecBC or RecBCD, where there is little net DNA unwinding (see below). There could also be protein–protein interactions between SSB and RecBC(D) which cause the stimulation we observe, although there is no direct evidence in support of this possibility.

Efficiency of DNA Unwinding Determined from Initial Rate Measurements. The apparent efficiencies of coupling between ATP hydrolysis and DNA unwinding by each enzyme are given in Table 1.[3] The RecBCD enzyme hydrolyzes about two ATP molecules for each DNA base pair unwound, while the ratios for the RecBC and RecBCD-K177Q enzymes are consistently smaller than those for RecBCD. The apparent efficiencies are about the same for both DNA substrates used and show no systematic dependence on the DNA concentration, as expected since the efficiency of a RecBC(D) enzyme/DNA complex should not depend on the concentration of those complexes. Most efficiency measurements were done with 200 μM ATP, a concentration well above the K_m's for ATP determined in steady-state measurements (Roman & Kowalczykowski, 1989b; Korangy & Julin, 1992b; Korangy & Julin, 1993). The efficiency did not change appreciably at higher ATP concentrations (efficiency = 2.34 and 2.11 ATP/bp (RecBCD enzyme), and 1.54 and 1.58 (RecBC), at 500 and 800 μM ATP, respectively). The efficiency we find for RecBCD is close to that reported previously for this enzyme (as few as 1.3–2 ATP/bp, depending on the reaction conditions, Roman & Kowalczykowski, 1989b). The Rep helicase from *E. coli* was also found by two different experimental approaches to hydrolyze about 2 ATP/bp (Kornberg et al., 1978; Yarranton & Gefter, 1979).

The apparent efficiency of unwinding by RecBCD and RecBC was greatly reduced at low ATP concentrations compared to 200 μM ATP. At 2 μM ATP (Figure 2) very little DNA unwinding is detected with either enzyme, although ATP is hydrolyzed. The observed efficiencies from Figure 2 are about 6 ATP/bp (RecBCD) and 20 ATP/bp (RecBC). RecBC hydrolyzed 4.8(±0.6) ATP/bp at 6 μM ATP and 2.4(±0.3) ATP/bp at 10 μM ATP (two determinations of each; data not shown). No ATP hydrolysis was seen in the absence of DNA or RecBC(D) enzyme in these experiments (data not shown). These results show that the RecBC(D) enzyme can bind to the DNA and hydrolyze ATP, at 2 μM, ATP, but that no substantial DNA unwinding takes place. It

[3] The values in Table 1 for the efficiencies are those calculated from rates of ATP hydrolysis and DNA unwinding measured in side-by-side determinations. The rates given are the overall averages of multiple determinations (2–20) in separate experiments.

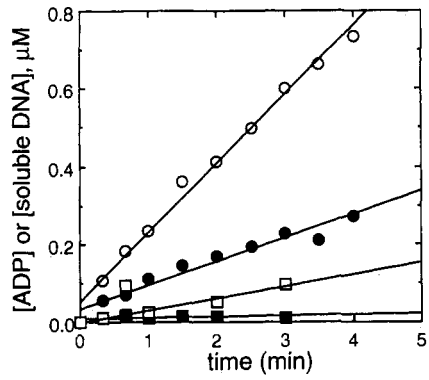


FIGURE 2: DNA unwinding and ATP hydrolysis at low ATP concentration. Reactions were done as in Figure 1 with pTZ19R [^{3}H]DNA linearized with *BglI* (30 μM bp), 2 μM ATP ([γ-^{32}P]-labeled or unlabeled), and 0.2 nM RecBCD enzyme (open symbols) or 0.2 nM RecBC enzyme (filled symbols). (circles) ADP concentration (μM); (squares) soluble DNA concentration (μM bp), determined by the RecJ/exoI coupled assay (see Experimental Procedures).

is possible that the enzyme unwinds a small amount of DNA at the end of the linear substrate but that the unwound DNA rewinds and is not detected by the coupling enzymes. We found previously that, at low ATP concentrations, both the RecBCD and RecBCD-K177Q enzymes are inefficient in coupling ATP hydrolysis to nucleolytic degradation of double-stranded DNA (Korangy & Julin, 1992b).

Biphasic ATP Hydrolysis in the Presence of SSB and High RecBC(D) Concentrations. The time courses of ATP hydrolysis are biphasic (Figure 3) in reactions containing higher concentrations of the RecBC(D) enzyme and lower DNA concentrations than those used in Figure 1B. A rapid reaction is followed by a much slower second phase. The amount of ATP hydrolyzed in the rapid reaction phase increases proportionally with the DNA concentration (Figure 3). Similar time courses have been observed before for these reactions (Roman & Kowalczykowski, 1989b; Korangy & Julin, 1992c).

The rapid ATP hydrolysis reaction corresponds to that required for complete unwinding of the DNA substrate. To examine the DNA in the RecBC reaction mixtures, samples were removed, added to SDS to remove the bound SSB, and analyzed on an agarose gel (Figure 4). Most of the DNA comigrates with a denatured DNA marker by 2 min, and all is unwound by 4–6 min. The rapid phase of ATP hydrolysis was complete by 4–6 min in this experiment (Figure 3A) and thus corresponds to the ATP hydrolysis required for complete unwinding of the double-stranded DNA substrate. In another experiment, about 88–93% of the DNA was sensitive to S1 nuclease in samples removed at 6, 10, 14, and 20 min from reactions with 20 μM (bp) [^{3}H]DNA and 1.2 nM RecBC enzyme, and 97–100% in reactions with 40 μM DNA, confirming that all of the DNA is unwound by the time the slow second phase sets in. The fact that the amount of ATP hydrolyzed in the rapid phase increases proportionally with the amount of DNA present is consistent with this interpretation.

The slow phase in the reactions in Figure 3 results from ATP hydrolysis stimulated by the single-stranded DNA produced by the RecBC(D) helicase activity but greatly inhibited by the bound SSB protein. The rate of the slow second phase with double-stranded DNA is similar to that in reaction mixtures containing heat-denatured DNA rather than double-stranded DNA and the same concentrations of RecBC(D), ATP, and SSB (data not shown). We detect no ATP hydrolysis by any of the RecBC(D) enzymes in the absence of DNA and none in the SSB protein, under the conditions such as those in Figure 3 (data not shown).

Efficiency Determined from the Amount of ATP Hydrolyzed during Complete Unwinding in the Presence of SSB Protein. The biphasic ATPase kinetics in Figure 3 suggested a second way to determine the efficiency. The efficiency is equal to the amount of ATP hydrolyzed in the rapid phase divided by the total DNA concentration. The amount of ATP hydrolyzed in the rapid phase was determined simply by extrapolating the slow linear phase back to the vertical axis (time = 0; see Figure 3). The efficiencies calculated in this way (Table 3) are quite similar to those determined from the initial rate measurements (Table 1).

The RecBCD enzyme is sufficiently processive to completely unwind and degrade pPvSm19 in the time course of Figure 3 (Roman et al., 1992; Korangy & Julin, 1992c). However, we found previously that both the RecBC and RecBCD-K177Q enzymes are less processive than the wild-type RecBCD enzyme (Korangy & Julin, 1992c, 1993). We found that RecBC hydrolyzes on average 2400 molecules of ATP for each time it binds to a double-stranded DNA molecule (Korangy & Julin, 1993). If this leads to unwinding of about 1700 bp (≈2400 ATP ÷ 1.4 ATP/bp), then only about half of a pPvSm19 molecule could be unwound by two RecBC enzymes working at each end of a DNA molecule. While some enzyme molecules will travel farther than this average processivity, it is still surprising that RecBC should be able to completely unwind the pPvSm19 DNA in these reactions. Either the RecBC enzyme is more processive than indicated in the heparin experiments or the enzyme is not prevented by bound SSB protein from reinitiating unwinding on a partially unwound DNA, as are the RecBCD (Taylor & Smith, 1985;

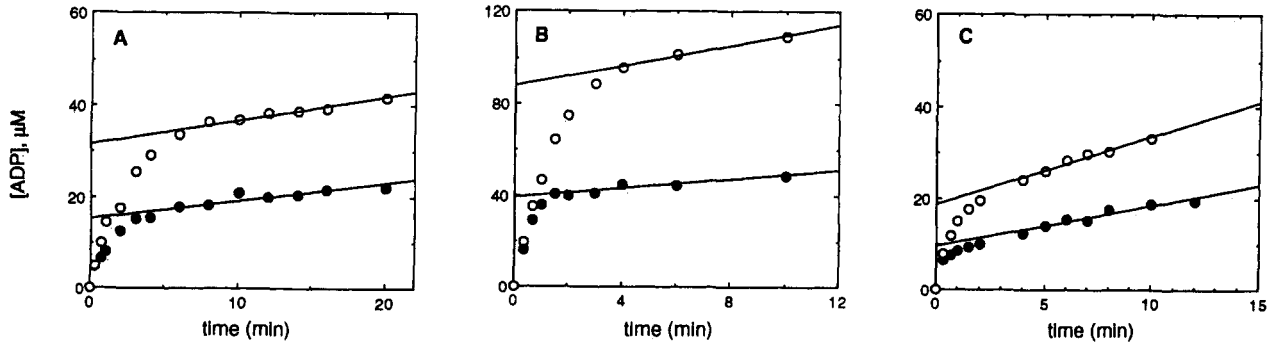


FIGURE 3: ATP hydrolysis during complete unwinding of double-stranded DNA in the presence of SSB. Reaction mixtures (standard conditions) contained 200 μM [γ-^{32}P]ATP, 16 μM SSB protein, and *Sac*I-cut pPvSm19 [^{3}H]DNA. Lines through the data are linear least squares fits of the second, slow, reaction phase. A. RecBC enzyme (1.6 nM), 10 (●) or 20 (○) μM DNA bp. B. RecBCD enzyme (0.645 nM), 17 (●) or 33 (○) μM DNA. C. RecBCD-K177Q enzyme (1.61 nM), 10 (●) or 20 (○) μM DNA.

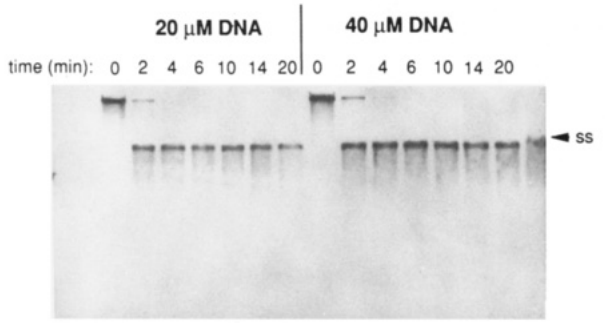


FIGURE 4: Agarose gel analysis of DNA unwinding by the RecBC enzyme. The reaction mixture was the same as in Figure 3A except the ATP was nonradioactive. The DNA concentrations were 20 and 40 μM nucleotides (10 and 20 μM bp). Samples were removed at the indicated times, quenched with 50 mM EDTA and 0.3% SDS, and loaded on a 0.7% agarose gel. The gel was treated with EN^3-HANCE, and the [^{3}H]DNA was visualized by autofluorography. The arrow indicates the position on the gel of a denatured DNA marker.

Table 3: Efficiency Measured from Complete Unwinding Experiments[a]

enzyme	DNA	size (bp)	efficiency (±s.e.)[b] (ATP/bp)		N
RecBCD	pPvSm19/*Sac*I	6250		1.80(0.12)	9
	pTZ19R/*Bgl*I	1595 and 1268		1.55(0.22)	3
			overall average:	1.74(0.10)	12
RecBC	pPvSm19/*Sac*I			1.29(0.07)	17
	pTZ19R/*Bgl*I			1.28(0.06)	18
			overall average:	1.28(0.05)	35
RecBCD-K177Q	pPvSm19/*Sac*I			1.09(0.11)	7
	pTZ19R/*Bgl*I			0.96	1
			overall average:	1.07(0.10)	8

[a] Reaction mixtures (standard conditions) contained 200 μM ATP, 10–40 μM DNA bp, 8 or 16 μM SSB protein, 0.32–3.2 nM RecBCD enzyme, 0.5–1.75 nM RecBC enzyme, or 1.6–3.6 nM RecBCD-K177Q enzyme. [b] The efficiency was calculated from the extrapolated intercept on the vertical axis of time courses such as those in Figure 3 (see Results) divided by the initial concentration of DNA base pairs.

Roman et al., 1992) and RecBCD-K177Q enzymes (Korangy & Julin, 1992c).

We attempted to determine the processivity of the RecBC enzyme by another experimental procedure, in order to resolve this apparent contradiction. We decided to look at the effect of adding a large amount of nonradioactive double-stranded DNA to reactions of RecBC(D) enzyme with a radiolabeled DNA substrate. We then examined the DNA on an agarose gel or added S1 nuclease to solubilize the unwound DNA. The logic of this trapping experiment is the same as that of the previous heparin experiments (Korangy & Julin, 1993), except that we are measuring production of single-stranded DNA directly rather than indirectly through ATP hydrolysis measurements. Attempts to do these unwinding measurements with heparin as the trap were unsuccessful, apparently because heparin inhibits both SSB and S1 nuclease.

Figure 5, lanes 2–6, shows that the RecBC enzyme is able to completely unwind pDJ01 DNA (21.4 kb) in the presence of SSB protein. The reaction contains a 2-fold excess of active RecBC enzyme over the DNA ends. However, only a small amount of pDJ01 is unwound when nonradioactive trap DNA (a 10-fold excess of pPvSm19 cleaved with *Bgl*I (100-fold excess of DNA ends)) is added along with ATP to start the reaction (Figure 5, lanes 7–11). This latter result shows that the extensive unwinding of pDJ01 in lanes 2–6 is not due to processive action by RecBC, since in that case the unwinding should not be affected by the trap DNA. Instead, the result in Figure 5, lanes 7–11, is as expected for a moderately processive enzyme.

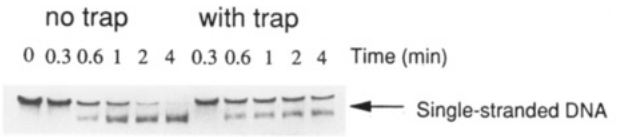


FIGURE 5: RecBC-catalyzed unwinding of pDJ01 DNA (21.4 kb). Lanes 1–6. Reaction mixtures (standard conditions) contained 200 μM ATP, pDJ01 [^{3}H]DNA linearized with *Kpn*I (10 μM bp, 0.93 nM ends), 8 μM SSB, and 1.8 nM RecBC enzyme. Lanes 7–11. Reaction mixtures were as in lanes 1–6, except that the reaction was initiated by adding a mixture of ATP (200 μM) and pPvSm19 DNA cleaved with *Bgl*I to produce five fragments of average size 1250 bp (100 μM bp; 160 nM ends). Sample treatment, agarose gel electrophoresis, and autofluorography were done as in Figure 4.

A similar conclusion can be drawn from the same experiment probed with S1 nuclease. The reaction of RecBC enzyme with pDJ01 [^{3}H]DNA in the presence of unlabeled trap DNA proceeds for a short time (20–40 s) but then slows considerably, to a rate about equal to that when both substrate and trap DNA are present from the start (Figure 6A). Most of the pDJ01 DNA becomes sensitive to S1 nuclease in the absence of trap. The amount of DNA made acid-soluble in this first 20 s of the reaction is about 2600 bp per DNA end, a value for the processivity in reasonable agreement with that estimated above from the heparin results (Korangy & Julin, 1993). The estimated rate of the unwinding in Figure 6A, 7800 bp/min (2400 nM bp/20 s, and assuming that there are 0.93 nM RecBC enzymes acting), is also in good agreement with unwinding rates for RecBC determined in the other experiments (Table 1).

The reaction of the RecBCD enzyme with pDJ01 [^{3}H]-DNA is virtually unaffected when excess trap DNA is added (Figure 6B). The same amount of unlabeled DNA added initially strongly inhibits the RecBCD enzyme-catalyzed reaction (Figure 6B). These results are consistent with the high processivity of the RecBCD enzyme (Roman et al., 1992). Control experiments (not shown) indicate that the amount of S1 nuclease added was able to solubilize 94% of the DNA, when both the [^{3}H]DNA and the pPvSm19 DNA were heat-denatured. Less than 1% of the double-stranded DNA was solubilized by the S1 alone. The SSB concentration was sufficient to bind to at least 30% of the maximum amount of single-stranded DNA which could be produced in the reaction, if *all* of the pDJ01 and pPvSm19 DNA were unwound, assuming an SSB monomer binds 8 nt of single-stranded DNA (Lohman & Overman, 1985).

We also did these trapping experiments with a smaller [^{3}H]-DNA substrate, pTZ19R, linearized with *Bgl*I to give two fragments of 1560 and 1297 bp. The reaction of RecBC with this substrate was unaffected by added unlabeled trap (Figure 6C), as expected if the enzyme is processive enough to completely unwind the shorter DNA substrate.

The simplest explanation for the results in Figures 5 and 6A is that the RecBC enzyme is moderately processive but that it is not prevented by SSB from reinitiating unwinding on a partially unwound DNA molecule. The DNA produced by RecBC would consist of a duplex terminated by two single-stranded, SSB-coated tails, due to the low nuclease activity of RecBC. SSB might not prevent RecBC from rebinding to this partially unwound DNA. However, partially degraded DNA molecules produced by the RecBCD and RecBCD-K177Q enzymes would probably be duplex molecules with only one SSB-coated, single-stranded tail under these reaction conditions. SSB might be more effective at preventing the RecBCD and RecBCD-K177Q enzymes from reinitiating the reaction on these DNA structures. Further study will be

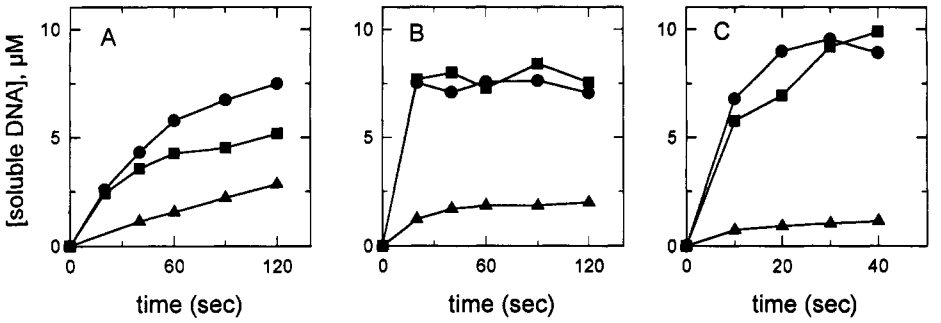


FIGURE 6: Effect of DNA trap on DNA unwinding by RecBC and RecBCD. All reaction mixtures (standard conditions) contained 200 μM ATP and 8 μM SSB. Aliquots were removed and treated with S1 nuclease as described in Experimental Procedures. A. Reaction mixtures contained pDJ01 [^{3}H]DNA linearized with *KpnI* (10 μM bp, 0.93 nM ends) and 1.8 nM RecBC enzyme. B. The reaction mixture was as in part A except it contained 3.7 nM RecBCD enzyme. C. The reaction mixture contained pTZ19R [^{3}H]DNA (0.75 μM bp, 1.0 nM ends) cleaved with *BglI* and 3 nM RecBC enzyme. (▲) Trap DNA (pPvSm19 DNA cleaved with *BglI* (100 μM bp; 160 nM ends)) was present initially in the reaction mixture. (■) The reaction was initiated by adding ATP and trap together. (●) No added trap.

required to completely understand the interaction between RecBC and SSB.

We found previously that the mutant RecBCD-K177Q enzyme is also less processive than the wild-type enzyme, by three different experimental measurements (Korangy & Julin, 1992c). In experiments quite analogous to those reported in Table 3, we measured the amount of ATP hydrolyzed by RecBCD-K177Q during one processive reaction with a 21.4 kb DNA molecule, in the presence of SSB protein. The result was that RecBCD-K177Q hydrolyzed about 3500 ATP per DNA end (see Table II in Korangy and Julin (1992c)). This result was interpreted as indicating the reduced processivity of RecBCD-K177Q compared to RecBCD, since the latter hydrolyzed 28 000 ATP per DNA end (Korangy & Julin, 1992c). Other experiments indicated that the SSB protein prevents the mutant RecBCD-K177Q enzyme from reinitiating on a partially unwound/degraded DNA substrate (Figure 4 in Korangy and Julin (1992c)), as it does with the wild-type enzyme (Taylor & Smith, 1985; Roman et al., 1992). If RecBCD-K177Q hydrolyzes 1.2 ATP/bp unwound (see Table 1 in this paper), then 3500 ATP hydrolyzed would correspond to about 2900 bp unwound. That is, two RecBCD-K177Q enzyme molecules acting from each end of a pPvSm19 molecule would, on average, be almost able to completely unwind that DNA. However, experiments in which S1 nuclease was used to measure directly the amount of DNA unwound by RecBCD-K177Q (with SSB included to trap the unwound DNA) gave a lower value for this enzyme's processivity (about 1250 bp unwound from each end of a large DNA substrate (Korangy & Julin, 1992c)). If this is a true estimate of the processivity of RecBCD-K177Q, then it should be unable to completely unwind pPvSm19, and the values for the efficiency of RecBCD-K177Q in Table 3 would then be an underestimate. The efficiency of the mutant enzyme is therefore somewhat uncertain, due to the uncertainty as to its precise processivity.

DISCUSSION

Efficiency of RecBCD, RecBC, and RecBCD-K177Q. We have used two experimental approaches to measure the efficiency of the RecBCD, RecBC, and RecBCD-K177Q enzymes. Similar results are obtained by each method. The RecBCD enzyme hydrolyzes about two ATP molecules for every base pair of DNA made single-stranded by its helicase and nuclease activities under our reaction conditions. The RecBC enzyme is at least as efficient as the RecBCD enzyme in coupling ATP hydrolysis to DNA unwinding. This enzyme is also capable of rapid DNA unwinding (within a factor of 4 of the wild-type enzyme under the reaction conditions used here (Table 1)). RecBC acts processively, as determined by heparin-trapping experiments (Korangy & Julin, 1993), although its processivity is also lower than that of the RecBCD enzyme. The RecBCD-K177Q enzyme is also capable of rapid and efficient DNA unwinding.

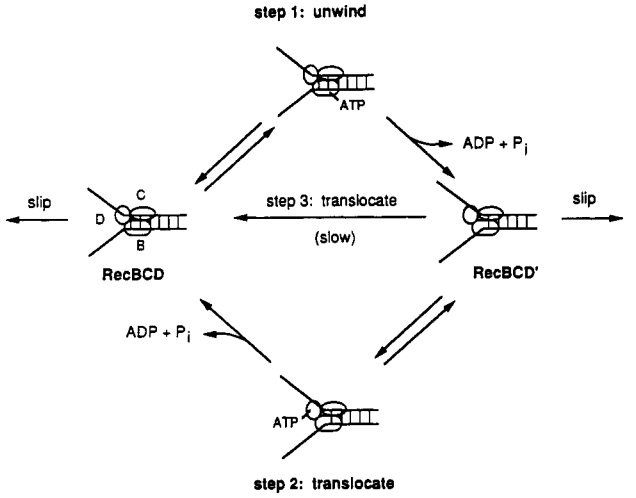
The catalytic properties of the RecBC enzyme show that the RecD subunit is dispensable for helicase activity by RecBCD. However, the fact that RecBC and RecBCD-K177Q hydrolyze less ATP while unwinding DNA than does the RecBCD enzyme indicates that the RecD subunit does hydrolyze ATP during the reaction catalyzed by the RecBCD enzyme. These results suggest that RecD must be a quite active ATPase subunit in the RecBCD holoenzyme, hydrolyzing about as much ATP as does the RecB subunit. The isolated RecD subunit has not been found to catalyze ATP hydrolysis in previous studies (Lieberman & Oishi, 1974; Masterson et al., 1992). RecD could require the presence of one or both of the other subunits to take on an active conformation or to bind effectively to the DNA.

The conclusion that RecD is an ATPase is supported by the effects of a site-directed mutation in the putative ATP-binding site of the RecB subunit (Hsieh & Julin, 1992). This RecB-mutant enzyme (RecB-K29Q-CD enzyme) is an ATPase with single-stranded DNA but not with blunt-ended double-stranded DNA. This finding suggests that the unaltered RecD subunit (in either the RecB-mutant enzyme or the RecBCD enzyme) is a DNA-dependent ATPase, but with single-stranded DNA only. The RecB-K29Q-CD enzyme is also an *ATP-dependent* nuclease on single-stranded DNA (Hsieh & Julin, 1992).

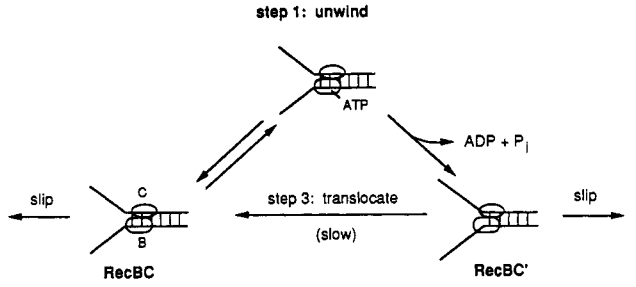
The high level of helicase activity we find for the RecBC enzyme is in agreement with recent experiments, indicating that the RecBC enzyme unwinds DNA *in vivo* in *recD* mutants (Rinken et al., 1992). Our findings are also interesting in light of one suggested function of Chi recombination hot-spot sequences. The nuclease activity of the RecBCD enzyme is suppressed after the enzyme encounters a Chi sequence in double-stranded DNA (Dabert et al., 1992; Dixon & Kowalczykowski, 1993). It has been proposed that the RecD subunit dissociates when the RecBCD enzyme interacts with a Chi sequence, leaving RecBC bound to and active on the DNA substrate (Thaler et al., 1988). Our results do not address directly the proposed loss of RecD, but they do show that the resulting RecBC enzyme would remain as a highly active helicase.

Helicase Reaction Mechanisms. The catalytic mechanism of a DNA helicase is thought to involve a reaction cycle

Scheme 1: RecBCD



Scheme 2: RecBC



dependent on ATP binding and hydrolysis (Hill & Tsuchiya, 1981; Lohman, 1992). Each ATP hydrolysis reaction is thought to be coupled to changes in the interactions between the helicase and single- and double-stranded DNA in the substrate, to bring about DNA unwinding. The number of DNA base pairs unwound during a single cycle (the step size) is not known for any DNA helicase. Helicase mechanisms have been proposed which require one ATP hydrolyzed per DNA base pair unwound (Hill & Tsuchiya, 1981), 2 ATP/bp (Yarranton & Gefter, 1979), or a nonintegral amount, proposed specifically for RecBCD (Roman & Kowalczykowski, 1989b). A mechanism advanced recently for the Rep helicase proposes that hydrolysis of one ATP enables multiple base pairs to be unwound (Wong & Lohman, 1992). The rest of the ATP hydrolyzed (to give a net of 2 ATP/bp unwound) is proposed to be catalyzed by Rep protein bound to single-stranded DNA produced by its helicase activity and is therefore uncoupled from DNA unwinding. ATP hydrolysis which does not result in net unwinding could also occur if the enzyme slips during DNA unwinding, as discussed by Hill and Tsuchiya (1981).

Mechanism of the RecBCD Enzyme. Schemes 1 and 2 show mechanisms for the RecBCD and RecBC enzymes consistent with the results reported in this paper and essentially the same as the one we proposed previously (Korangy & Julin, 1993) on the basis of studies of the RecBC, RecBCD, and RecBCD-K177Q and RecB-K29Q-CD mutant enzymes (Korangy & Julin, 1992b,c, 1993; Hsieh & Julin, 1992). The mechanism proposes that DNA unwinding and translocation are two different steps of the reaction and that ATP hydrolysis by the RecD subunit functions in the translocation step. An additional possible function for RecD in the nuclease activity is also discussed below.

We believe that DNA unwinding catalyzed by the RecBCD holoenzyme or by RecBC is initiated by ATP hydrolysis catalyzed by the RecB subunit (step 1, Schemes 1 and 2). The isolated RecB protein hydrolyzes ATP with single- or double-stranded DNA, and it is able to unwind short stretches of duplex DNA (Boehmer & Emmerson, 1992). Thus it is clear that RecB catalyzes ATP hydrolysis coupled to DNA unwinding in the RecBC and RecBCD enzymes.

As discussed above, the RecD subunit in the RecBCD holoenzyme appears to be an ATPase stimulated by single-stranded DNA. We proposed that ATP hydrolysis by RecD (step 2, Scheme 1) occurs after ATP hydrolysis catalyzed by RecB, at the intermediate called RecBCD′. This enzyme/DNA complex is postulated to occur after the unwinding step but before translocation, and ATP hydrolysis catalyzed by RecD is favored over that by RecB in RecBCD′ (Korangy & Julin, 1992c, 1993). We suggest that ATP hydrolysis catalyzed by the RecD subunit stimulates the movement of RecD (and RecBCD) along the DNA unwound in step 1. This function for RecD could contribute to the rapid and highly processive helicase activity of the RecBCD enzyme, but RecD would not be *required* for DNA unwinding. We do not have a direct measurement of the step size (see above) for the RecBCD enzyme. Scheme 1 predicts that RecBCD should hydrolyze 2 ATP/bp, about what we observe, if we assume that the step size is one base pair.

Scheme 2 is the mechanism which we believe applies to the RecBC enzyme. The DNA unwinding step (step 1) remains dependent on ATP hydrolysis by RecB. However, this enzyme lacks the RecD- and ATP-driven translocation step (step 2). Instead, step 3 in Scheme 2 (and 1) represents hypothesized ATP-independent (and RecD-independent) translocation which must occur in both the RecBC and RecBCD-K177Q enzymes. If this movement is slower than the ATP-dependent movement via step 2, then the RecBC and RecBCD-K177Q enzymes will be slower than RecBCD enzyme, as observed.

Scheme 1 also allows for the possibility of slippage. The RecBCD enzyme could sometimes hydrolyze an ATP molecule and unwind a base pair but then slip back and allow the unwound DNA to reanneal, rather than advancing to the next base pair. However, our interpretation of the measured efficiency for the RecBCD enzyme of 2 ATP/bp is that these are hydrolyzed for the two steps in the unwinding/translocating process. Implicit in this interpretation is that the enzyme does not slip often and that no ATP is wasted due to slippage. The frequency of slipping by the RecBCD enzyme before either step 1 or step 2 would depend on the relative rate of step 1 or step 2 compared to the slipping rate. The rates of steps 1 and 2 are high at high ATP concentrations, and so Scheme 1 predicts that the RecBCD enzyme would not slip often at high ATP, consistent with our interpretation. However, the low efficiency observed at 2 μM ATP can be explained if the ATP-dependent steps become slow, and so slippage is more likely to occur than at a high ATP concentration.

Slippage by RecBC rather than unwinding (step 1) should also be of little significance at high ATP concentrations, by the same reasoning as above. However, the observed efficiency for RecBC of about 1.3–1.4 ATP/bp can be explained if RecBC hydrolyzes 1 ATP/bp (step 1) but slips about 30–40% of the time at RecBC′. The higher probability of slipping by RecBC could be a consequence of ATP-independent translocation (step 3) being slower for RecBC than is the RecD-dependent translocation (step 2) available to RecBCD. If this is true, than the overall coupling between ATP hydrolysis and DNA unwinding is actually less efficient for RecBC than for RecBCD.

Role for RecD in the Nuclease Reaction. The proposal that RecD is an ATP-dependent translocation subunit is consistent with the reductions in the rate and processivity of the unwinding reaction catalyzed by the RecBC enzyme compared to RecBCD. The RecD subunit also appears to function in the nuclease activity, as was concluded from the originally isolated *recD* mutants (Chaudhury & Smith, 1984). Indeed, while the helicase activity of RecBCD is reduced 4-fold when RecD is lost, the nuclease activity (measured by degradation of double-stranded DNA to small fragments) is essentially completely abolished (Palas & Kushner, 1990; Masterson et al., 1992; Korangy & Julin, 1993). The fact that the RecB-K29Q-CD mutant enzyme retains ATP-dependent nuclease activity on linear single-stranded DNA (Hsieh & Julin, 1992) suggests that ATP hydrolysis by RecD may be important for stimulating this nuclease activity. The stimulation could be a consequence of the translocation function postulated for RecD, or the nuclease cleavage reaction could be directly coupled to ATP hydrolysis by RecD. We note that the RecBCD-K177Q mutant enzyme retains nuclease activity on both single- and double-stranded DNA (Korangy & Julin, 1992b), although its activity is reduced compared to that of the wild-type enzyme. Thus the presence of the RecD subunit, and not necessarily its ATP hydrolysis activity, appears to enable the enzyme to degrade double-stranded DNA. Further work will be required to elucidate the precise role of RecD in the nuclease reaction.

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