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On the Role of ATP in Phosphodiester Bond Hydrolysis Catalyzed by the *rec*BC Deoxyribonuclease of *Escherichia coli**

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The deoxyribonuclease specified by the *rec*B and *rec*C genes of *Escherichia coli* (*rec*BC DNase; exonuclease V) has been purified to near homogeneity by a new procedure. Although hydrolysis of even a single nucleotide from a duplex DNA molecule by the pure enzyme is absolutely dependent upon ATP, the extent of phosphodiester hydrolysis is strongly inhibited by ATP concentrations of 0.2 mM or greater, and the initial rate is unaffected. Under these conditions, the extent of DNA hydrolysis is proportional to enzyme concentration. In contrast, neither the rate nor the extent of hydrolysis of single-stranded DNA nor ATP is affected by high concentrations of ATP.

The amount of large single-stranded polynucleotide generated by the action of the *rec*BC DNase increases as the ATP concentration increases and, at 0.5 mM ATP, becomes equivalent to the amount of acid-soluble nucleotide formed.

These findings suggest that high intracellular concentrations of ATP affect the mechanism of the *rec*BC DNase so as to limit the extent of hydrolysis of duplex DNA, while at the same time favoring the formation of single-stranded regions within the duplex. Such regions may be essential intermediates in the recombination process.

The deoxyribonuclease specified by the *rec*B and *rec*C genes (*rec*BC DNase) is required for normal genetic recombination in *Escherichia coli*. The enzyme has been purified by Linn and co-workers (1) and has been shown to consist of two subunits of molecular weight 140,000 and 128,000. The *rec*BC DNase catalyzes the exonucleolytic hydrolysis of linear duplex and single-stranded DNA in reactions that are absolutely dependent on ATP (1-4). Optimal rates of hydrolysis of single- and double-stranded DNA occur at different ATP concentrations, suggesting that the function of ATP in the reaction catalyzed by the *rec*BC DNase may be complex. During the exonucleolytic hydrolysis of DNA, ATP is hydrolyzed to ADP and P_i in amounts that can exceed considerably the number of phosphodiester bonds cleaved (1-4). Under certain conditions (2), hydrolysis of ATP can occur without phosphodiester bond cleavage.

As part of an effort to define the role of the *rec*BC nuclease in recombination, we have developed a new and simplified purification procedure that leads to homogeneous enzyme in reasonable yield. With this purified enzyme we have undertaken an investigation of the role of ATP in phosphodiester bond hydrolysis. These studies have demonstrated that the extent of reaction as well as the nature of the products formed can be modulated by ATP concentration.

EXPERIMENTAL PROCEDURES

Materials

S_1 nuclease was purchased from Miles Laboratories. One unit of enzyme is the amount that produces 1 μg of acid-soluble nucleotide in 30 min from single-stranded DNA at 37°. Lysozyme (salt-free) was purchased from Worthington. *Escherichia coli* alkaline phosphatase, free of deoxyribonuclease, was kindly provided by Dr. Stuart Linn (University of California, Berkeley) and *E. coli* DNA polymerase I was a gift from Dr. Arthur Kornberg (Stanford). T4 polynucleotide kinase was purified by the procedure of Richardson (5).

^{3}H- and ^{32}P-labeled T7 DNAs were prepared according to the procedure of Richardson (6). The specific radioactivities were 6-10 × 10^3 and 2 × 10^4 cpm per nmol, respectively. Dr. Robert Bombara (Stanford) kindly provided the ϕ80 DNA and ϕ80 DNA in which the cohesive ends were filled in with [^{32}P]dCTP (2 to 10 × 10^7 cpm/nmol) and the other three unlabeled deoxynucleoside triphosphates. Synthesis of ϕ80 DNA with ^{3}H-labeled nucleotides at the 3′ termini and ^{32}P at the 5′ termini was accomplished in the following manner. The cohesive ends were filled in using *E. coli* DNA polymerase I and [^{3}H]dGTP (2.5 × 10^7 cpm/nmol) together with the other unlabeled deoxynucleoside triphosphates, as described by Wu *et al.* (7), to yield DNA labeled with ^{3}H at the 3′ termini. The 5′ termini were then labeled with ^{32}P by treatment with bacterial alkaline phosphatase, followed by T4 polynucleotide kinase and [γ-^{32}P]ATP (4.5 × 10^4 cpm/pmol) according to the method of Richardson (6).

ATP was purchased from P-L Biochemicals. [γ-^{32}P]ATP was prepared by the method of Glynn and Chapell (8) and purified by DEAE-Sephadex A25 (HCO_3^-) chromatography according to the procedure of Schendel and Wells (9). [α-^{32}P]dCTP and [^{3}H]dGTP were purchased from New England Nuclear. Calf thymus DNA (grade A) was purchased from Calbiochem. DNA concentrations are expressed as moles of nucleotide. Bovine serum albumin, dithiothreitol, and spermidine trihydrochloride were purchased from Calbiochem. DEAE-cellulose DE52 and phosphocellulose P11 were obtained from Whatman. Agarose A-1.5m (200 to 400 mesh) was purchased from Bio-Rad Laboratories. DNA-cellulose was prepared by the method of Alberts *et al.* (10). "Ultra-pure" ammonium sulfate and sucrose were obtained from Schwarz/Mann Research Laboratories.

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Methods

Assay of recBC DNase – The assay measures conversion of native T7 DNA to acid-soluble nucleotides. The reaction mixture (0.3 ml) contained 50 mM Tris/HCl (pH 8.5), 10 mM $MgCl_2$, 0.67 mM dithiothreitol, 1 mg/ml of albumin, 5 nmol of T7 [^{3}H]DNA, 25 to 500 μM ATP, and 0.3 to 1 unit of enzyme diluted in a solution composed of 10 mM potassium phosphate (pH 6.7), 0.1 mM dithiothreitol, 0.1 mM EDTA, 1 mg/ml of albumin, and 20% glycerol. After 20 min at 37°, the mixture was chilled and 0.2 ml of 0.6 mM calf thymus DNA and 0.3 ml of 15% trichloroacetic acid were added. Acid-insoluble material was removed by centrifugation at 20,000 × *g* at 2° for 15 min. The radioactivity of 4 ml of supernatant fluid was determined by mixing with 4 ml of Triton X-100 scintillation fluid (1 liter of Triton X-100, 2 liters of toluene, 4 g of 2,5-diphenyloxazole, and 0.1 g of 1,4-bis[2-(5-phenylazolyl))benzene]. One unit of enzyme is that amount which produces 1 nmol of acid-soluble nucleotide in 20 min.

Other Methods – Hydrolysis of [γ-^{32}P]ATP was measured according to Goldmark and Linn (1). Protein was determined by the method of Lowry *et al.* (11) with albumin as a standard.

Purification of recBC DNase – Unless otherwise specified, all operations were carried out at 0° to 4°, and all centrifugations were at 20,000 × *g* for 15 min. Six hundred grams of cell paste was used in a typical preparation.

Growth and Storage of Cells – *E. coli* strain JC4583 was grown in LB medium (10 g of Bacto-tryptone, 5 g of Bacto-yeast extract, 10 g of NaCl/liter of tap water) supplemented with 0.5% glucose. Growth was at 37° with vigorous aeration to approximately 10^9 cells/ml (under these conditions the culture was still in log phase). The cells were harvested, and the cell paste was washed with 50 mM Tris/HCl (pH 7.5), 0.1 M NaCl at room temperature, and resuspended in Buffer A (50 mM Tris/HCl (pH 7.5), 10% sucrose) such that 5 μl of cell suspension added to 2 ml of H_2O gave an A_{595} of 0.55. The cell suspension was divided into 20-ml aliquots and quickly frozen in liquid N_2. The cell suspension could be stored at −20° for several months.

Lysis – Cell extracts were prepared using a modification of the procedure of Wickner *et al.* (12). The frozen cell suspension was thawed slowly at 4° and then made 0.2 mg/ml in lysozyme. After incubation for 5 min at 0°, 4 M NaCl and 0.4 M spermidine were added such that their final concentrations in the cell suspension were 0.1 M and 0.01 M, respectively. After incubation for 25 min at 0°, the suspension was warmed to 20° in a 37° bath (approximately 90 s were required). As soon as the temperature reached 20°, the suspension was chilled rapidly to 0° and centrifuged for 60 min. The supernatant fluid (1090 ml) was collected (Fraction I).

Ammonium Sulfate Fractionation – Solid ammonium sulfate (29 g/100 ml) was added slowly to Fraction I by stirring which was continued for 30 min after all the salt had dissolved. The precipitate was collected by centrifugation, and the pellet was resuspended in 1/4 volume (Fraction I) of 45% saturated ammonium sulfate in Buffer B[1] (20 mM potassium phosphate (pH 6.7), 1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol). After a uniform homogenate was obtained, the precipitate was collected by centrifugation. This procedure was repeated using 1/10 volume (Fraction I) successively of a 40, 35, and 30% saturated ammonium sulfate solution in Buffer B. Typically, more than 70% of the enzyme activity was obtained by extraction with 30% saturated ammonium sulfate; however, the activity of the 35% extract was measured routinely, and the two fractions pooled if there was significant activity in the 35% extract. Solid ammonium sulfate was added to 60% saturation, and the precipitate was collected by centrifugation. The pellet was resuspended in Buffer B (60 ml) (Fraction II).

DEAE-cellulose Chromatography – A DEAE-cellulose column (2.5 × 40 cm) was equilibrated with Buffer B. Fraction II was diluted with Buffer B so that the conductivity was equivalent to 0.15 M KCl in Buffer B and was applied to the column at a rate of 30 ml/h. The column was washed with 1 bed volume of 0.15 M KCl in Buffer B, and a 5-bed volume linear gradient from 0.15 M to 0.5 M KCl in Buffer B was applied. Enzyme activity was eluted between 0.3 and 0.35 M KCl in Buffer B. Peak fractions were pooled and precipitated by the addition of 0.39 g/ml of solid ammonium sulfate. The pH was maintained near neutrality during the precipitation by dropwise addition of 1 M NaOH. The precipitate was collected by centrifugation and resuspended in a minimal volume of Buffer C (20 mM potassium phosphate (pH 6.7), 0.1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol) (6 ml) (Fraction III).

Agarose A-1.5m Filtration – Agarose A-1.5m (200 to 400 mesh) was equilibrated with Buffer C. A column size (4 × 95 cm) was chosen such that the volume of sample represented 3% of the column bed volume. Fractions were collected at a rate of 0.25 ml/min and the active fractions were pooled (68 ml) (Fraction IV).

DNA-cellulose Chromatography – A 3-ml DNA-cellulose column was washed with 100 bed volumes of Buffer D (20 mM potassium phosphate (pH 8.0), 0.1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol). Fraction IV was made 0.05 M in KCl and the pH was adjusted to 8.0 with 1 M KOH. After the sample was applied to the column, it was washed with 2 bed volumes each of 0.05 M, 0.15 M, 0.25 M, 0.35 M, 0.7 M, and 1 M KCl in Buffer D. Most of the activity applied was eluted by 0.7 M KCl. Peak fractions were pooled (Fraction V, 15 ml) and dialyzed against Buffer E (10 mM potassium phosphate (pH 6.7), 0.1 M EDTA, 0.1 mM dithiothreitol, 20% glycerol). Fraction V was concentrated to 1.0 ml by dialysis against dry Sephadex.

Phosphocellulose – Fraction V was applied to a 1-ml column of phosphocellulose equilibrated with Buffer E. Enzyme activity appeared in the pass-through fraction (Fraction VI) (1.4 ml).

Glycerol Gradient Sedimentation – A 20 to 40% glycerol gradient in Buffer E was prepared as described by Goldmark and Linn (1). A 0.25 ml-sample was layered onto a 5-ml gradient and centrifuged at 50,000 rpm for 15 h in an SW 50.1 rotor at 4°. Peak fractions were pooled and stored in liquid N_2 (Fraction VII).

Fraction VII (3 ml) contained greater than 90% pure *rec*BC DNase as judged by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and represented a 25,000-fold purification over Fraction I (Table I). The purified protein consisted of two subunits with molecular weights of 130,000 and 120,000, values which are in good agreement with those reported by Goldmark and Linn (1). All studies to be described were performed using Fraction VII.

RESULTS

Effect of ATP Concentration on Kinetics of Hydrolysis of Duplex DNA by recBC DNase – Hydrolysis of duplex DNA by the *rec*BC DNase is dependent upon ATP, which is cleaved to yield ADP and P_i (1–4). In fact, ATP is required for the hydrolysis of even a single nucleotide. Thus, when ϕ80 DNA, with approximately 5 [^{32}P]dCMP residues at the 3′ ends of the molecule was treated with the *rec*BC DNase, less than 0.1% of the ^{32}P was made acid-soluble in the absence of ATP (Table II).

The effect of ATP concentration on the rate of hydrolysis of duplex T7 DNA is shown in Fig. 1. Although the initial rate (up to 5 min) was unaffected by ATP concentration in the range of 25 to 100 μM, the extent of hydrolysis was strongly influenced by ATP concentration, and at 500 μM was proportional to enzyme added (Fig. 2). In confirmation of the reports of Goldmark and Linn (1) and Oishi (3), hydrolysis of single-stranded DNA by the *rec*BC DNase proceeded linearly for more than 20 minutes at both 25 and 500 μM ATP (Fig. 3). Similarly, the rate and extent of ATP hydrolysis in the presence of duplex T7 DNA were the same at both ATP concentrations (Fig. 4).

Irreversible Inactivation of the recBC DNase at 500 μM ATP – The basis for the limited extent of reaction at 500 μM ATP was investigated in the following experiments.

The *rec*BC DNase was preincubated with 500 μM ATP under standard assay conditions, but in the absence of DNA. Samples of the mixture were then assayed, at 25 μM and at 500 μM ATP. The ratio of activity for the two ATP concentrations was independent of the time of preincubation at 500 μM ATP. Hence, the decrease in the extent of reaction at 500 μM ATP is not the result of inactivation of the enzyme in the absence of catalysis (Table III).

To test whether the limited extent of reaction at 500 μM ATP is a result of the generation of products which inhibit the enzyme, the reaction was permitted to reach a plateau; then a

[1] Addition of 0.706 g of ammonium sulfate to 1 ml of Buffer B yielded a saturated solution at 0°.

fresh aliquot of enzyme was added. There was an immediate increase in the rate, identical to that seen initially (Fig. 5). Thus, the limited extent of reaction at high ATP concentrations does not result from the generation of a readily dissociable inhibitor. When aliquots were taken from a reaction mixture containing 500 μM ATP and reassayed at 25 μM ATP, there was no increase in activity, indicating that the apparent inactivation of the enzyme at 500 μM ATP cannot be reversed by lowering the ATP concentration (Fig. 6).

Effect of ATP Concentration on Products of Hydrolysis of Duplex DNA – S_1 nuclease, which specifically attacks single-stranded DNA, was used to determine the relative amount of single-stranded DNA generated by the action of *rec*BC DNase at various ATP concentrations. As shown in Fig. 7, the amount of acid-insoluble single-stranded DNA formed was dependent upon the ATP concentration, and at 500 μM ATP was nearly equivalent to the amount of acid-soluble nucleotide. Thus, the ATP concentration affected not only the extent of reaction but also the nature of the products.

TABLE I

Purification of recBC DNase

Fraction	Volume	Total units ($\times 10^4$)	Total protein	Protein
	ml		*mg*	*units/mg*
I. Extract	1090	19.0	12,300	8.4
II. Ammonium sulfate	60	7.3	1,000	55
III. DEAE-cellulose	6	4.2	48	868
IV. Agarose A-1.5m	68	4.5	4	11,200
V. DNA-cellulose	15	2.9		
VI. Phosphocellulose	1.4	1.11	0.11	101,000
VII. Glycerol gradient	3	1.0	0.05	218,000

The *rec*BC DNase can attack DNA at both the 3′ and 5′ termini of a duplex DNA molecule (2). We therefore wished to determine whether this bipolar mode of degradation was influenced by ATP concentration. Duplex ϕ80 DNA labeled at the 5′ end with ^{32}P and at the 3′ end with ^{3}H was incubated with the *rec*BC DNase at 25 μM and 500 μM ATP. Nucleotides were released from both termini at high and low ATP concentrations (Table IV), indicating that the bipolar mode of attack is not significantly affected by ATP concentration.

DISCUSSION

Our modification of the purification procedure of Goldmark and Linn (1) leads easily and reproducibly to nearly homogeneous *rec*BC DNase in reasonable yield. Our studies with the purified enzyme confirm previous reports that ATP is required (1–4) for the hydrolysis of linear duplex DNA. We have further

TABLE II

Hydrolysis of 3′ terminally labeled ϕ80 DNA by recBC DNase

Reaction mixtures (1 ml) contained 0.05 M Tris/HCl (pH 8.6), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 1 mg/ml of serum albumin, 3.26 nmol of ϕ80 [3′-^{32}P]DNA (1.07×10^4 cpm/pmol), 15 units of *rec*BC DNase, and where indicated, 50 μM ATP. At the times indicated, 0.15-ml aliquots were removed, precipitated, and processed as described under "Methods."

Time	Nucleotide made acid-soluble	
	+ATP	−ATP
min	*nmol*	
15	2.42	0.016
30	2.54	0.018
60	2.58	0.022
120	2.68	0.072

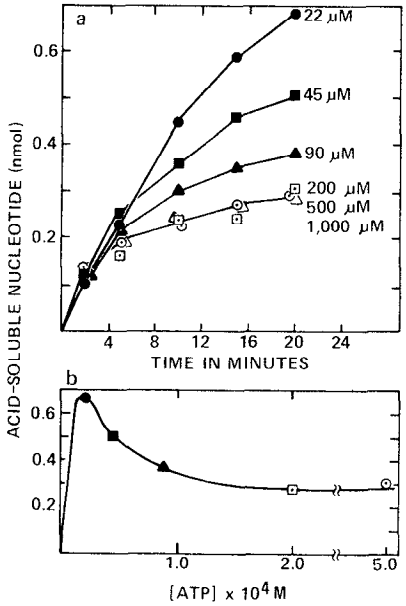

FIG. 1 (*left*). Effect of ATP concentration on hydrolysis of duplex DNA by *rec*BC DNase. The reaction mixtures (0.6 ml) contained 0.05 M Tris/HCl (pH 8.5), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 5 nmol of T7 [^{3}H]DNA (11.2×10^3 cpm/nmol), 1 mg/ml of serum albumin, and ATP as indicated. Reactions were started by the addition of 0.66 unit of enzyme, then aliquots (0.08 ml) were removed at the indicated times, added to 0.42 ml of 0.28 mM calf thymus DNA at 0°, and precipitated with 0.3 ml of 15% trichloroacetic acid. The precipitated samples were then processed as described for the standard assay (see "Methods"). *b* is a replot of the 20-min points of *a* as a function of ATP concentration.

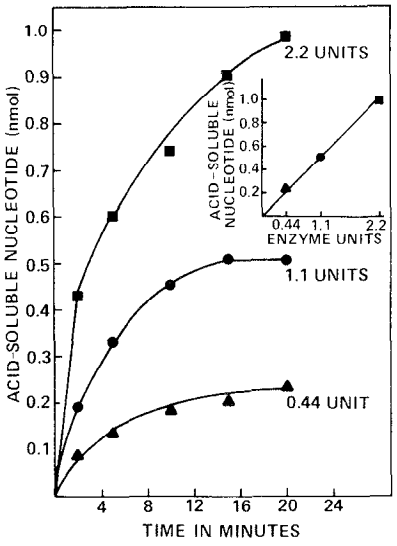

FIG. 2 (*center*). Effect of concentration of *rec*BC DNase on the rate and extent of hydrolysis of duplex DNA at 500 μM ATP. Assays were performed as described in the legend to Fig. 1, except that the amount of enzyme was varied as indicated and the concentration of ATP was 500 μM. The *inset* represents a plot of the 20-min points as a function of enzyme level.

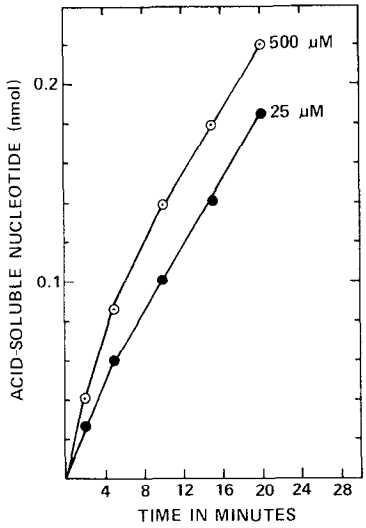

FIG. 3 (*right*). Effect of ATP concentration on hydrolysis of single-stranded DNA by *rec*BC DNase. The procedure was the same as that described in the legend to Fig. 1, except that heat-denatured T7 [^{3}H]DNA was used as the substrate and ATP concentrations were as indicated.

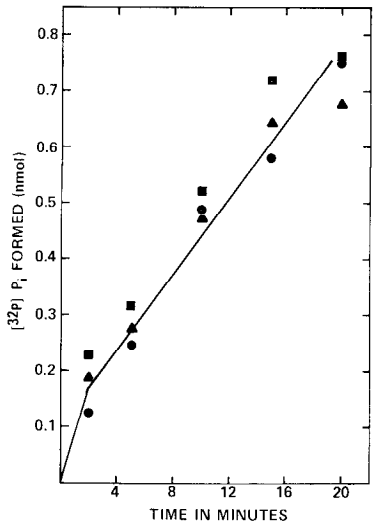

FIG. 4. Effect of ATP concentration on the hydrolysis of ATP by the *rec*BC DNase. Assays were as described under "Methods" except that the concentration of ATP was as indicated: ●, 25 μM ATP; ▲, 100 μM ATP; ■, 500 μM ATP.

TABLE III

Failure to inactivate recBC DNase by preincubation with 500 μM ATP in the absence of DNA hydrolysis

Incubation mixtures (0.05 ml) contained 0.05 M Tris/HCl (pH 8.9), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 1 mg/ml of serum albumin, 11 units of enzyme, and 500 μM ATP. At the times indicated, duplicate 5-μl aliquots were assayed in a mixture (0.6 ml) identical to that described above except that the concentration of ATP was 25 μM or 500 μM respectively, and contained 8.6 nmol of T7 [^{3}H]DNA (specific activity 11.3 × 10^3 cpm/nmol). Aliquots (0.08 ml) from the second reaction were withdrawn at the times indicated and assayed for acid-soluble nucleotide as described under "Methods."

First incubation	Second incubation (min)				
	2	5	10	15	20
min	*Activity ratio: 500 μM ATP/25 μM ATP*				
0	1.32	0.73	0.66	0.60	0.53
5	1.01	0.66	0.67	0.54	0.55
10	1.19	0.85	0.75	0.64	0.54

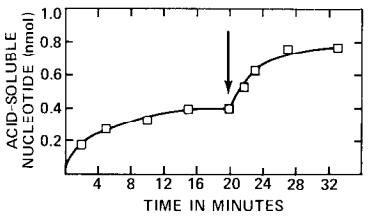

FIG. 5. Effect of further addition of *rec*BC DNase on hydrolysis of duplex DNA at 500 μM ATP. Assays (0.6 ml) were as described in the legend to Fig. 1 except the ATP concentration was 500 μM and 1.1 units of enzyme were added at 0 and 20 min.

shown that ATP is essential for the cleavage of even a single nucleotide from the 3′ terminus of a duplex DNA molecule. Although our findings substantiate the earlier observations that exonuclease activity is inhibited at high (500 μM) ATP concentrations (1, 3), we have found that the extent rather than the initial rate of the reaction is limited. Furthermore, the extent is proportional to the concentration of enzyme. This effect is very likely the result of inactivation of the enzyme as the reaction proceeds. Inactivation does not occur when single-

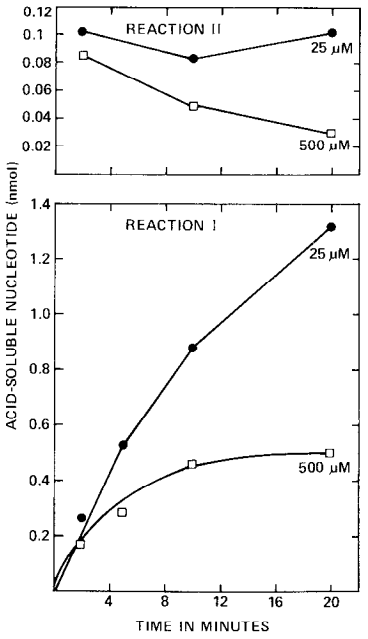

FIG. 6. Irreversibility of inactivation of *rec*BC DNase at 500 μM ATP. The reaction mixtures (0.2 ml) contained 0.05 M Tris/HCl (pH 8.5), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 10 nmol of T7 [^{3}H]DNA (11.2 × 10^3 cpm/nmol), 1 mg/ml of serum albumin, 4.4 units of enzyme, and either 25 μM or 500 μM ATP. Aliquots (0.01 ml) were removed at the times indicated and precipitated as described under "Methods" (*Reaction I*). Aliquots (0.01 ml) were also taken at 2, 10, and 20 min and assayed in a second reaction mixture (0.2 ml) identical to that described above except that the concentration of ATP was 25 μM. Aliquots (0.08 ml) were taken at 0 and 20 min, and the data in the *inset* (*Reaction II*) represent nucleotide made acid-soluble in the 20-min incubation period. The concentrations of ATP present in the first reaction mixture, are indicated by ●——●, 25 μM ATP; □——□, 500 μM ATP.

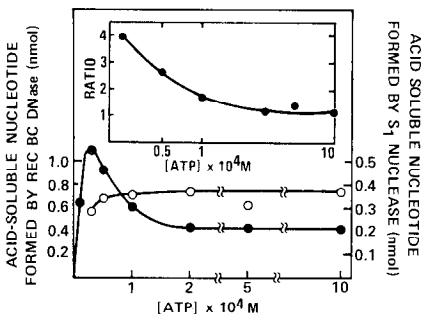

FIG. 7. Identification of products of *rec*BC DNase action on duplex DNA at increasing ATP concentrations. The 0.1-ml reaction mixtures contained 0.025 M Tris/HCl (pH 8.9), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 5 nmol of T7 [^{3}H]DNA (11.2 × 10^3 cpm/nmol), 1 unit of enzyme, and the concentrations of ATP as indicated. After 20 min at 37°, an aliquot (0.15 ml) was removed from each reaction and precipitated as described under "Methods." A second aliquot (0.15 ml) was made 0.05 M in sodium acetate, pH 4.6, 1 mM in $ZnCl_2$, 0.1 M in NaCl, and the volume adjusted to 0.2 ml. S_1 nuclease (2 × 10^3 units) was added to each reaction, and the mixture was incubated for 10 min at 37°. A 0.4-ml solution containing 0.25 mg/ml of serum albumin and 0.3 mM calf thymus DNA was added followed by 0.4 ml of 15% trichloroacetic acid. After 10 min at 0°, the precipitate was collected and the radioactivity of 0.6 ml of the supernatant fluid was determined. The amount of acid-soluble nucleotide formed by the action of the *rec*BC DNase alone (●——●) was subtracted from that resulting from the action of S_1 nuclease after incubation with *rec*BC DNase (○——○). The values on the ordinate in the *inset* refer to the ratio: acid-soluble nucleotide formed by *rec*BC nuclease to acid-soluble nucleotide formed by S_1 nuclease.

TABLE IV

Hydrolysis of T7 DNA from the 3′ and 5′ termini by recBC DNase

Reaction mixtures (0.75 ml) contained 0.05 M Tris/HCl (pH 8.6), 0.01 M $MgCl_2$, 0.67 mM dithiothreitol, 1 mg/ml of serum albumin, 10.9 nmol of ϕ80 [3′-^{3}H, 5′-^{32}P]DNA. (Specific activity of ^{3}H-labeled nucleotides, 2.3×10^2 cpm/nmol; specific activity of ^{32}P-labeled nucleotides, 1.9×10^2 cpm/nmol), 0.88 unit of enzyme, and ATP as indicated. Aliquots (0.15 ml) were removed at the times indicated and assayed for acid-soluble nucleotide as described under "Methods."

Time	Nucleotide made acid-soluble			
	25 μM ATP		500 μM ATP	
	3′-^{3}H	5′-^{32}P	3′-^{3}H	5′-^{32}P
min	*nmol*			
2	0.45	0.12		0.26
5	0.39	0.14	0.27	0.17
10	0.41	0.14	0.49	0.28
20	0.82	0.26	0.32	0.22

stranded DNA is used as a substrate, nor is the ATPase activity of the enzyme affected by high ATP concentrations. Thus, the DNase and ATPase activities of the *rec*BC enzyme are uncoupled during the latter stages of a reaction at high ATP concentrations. This behavior is analogous to that observed when RNA-DNA hybrids or DNA cross-linked with psoralen are used as substrates (2) or when duplex DNA is attacked in the presence of DNA binding protein (13).

The nature of the products changes as the concentration of ATP is increased. At high concentrations of ATP, the amount of single-stranded polynucleotide formed is increased to the point where it is nearly equivalent to the level of acid-soluble nucleotide. Thus, there appears to be preferential degradation of one of the two strands of the duplex at high ATP concentrations. Since the polarity of attack, at least initially, is unaffected by ATP concentration (2), degradation can occur from either the 5′ or 3′ end.

A plausible explanation of these findings is that at high ATP concentrations, the enzyme binds at the terminus of one of the strands of a duplex DNA molecule, hydrolyzing that strand to generate acid-soluble fragments. As a result, the number of acid-soluble nucleotides formed is equal to the number of nucleotides in the opposing strand that remains undegraded, or is degraded to only a limited extent. As hydrolysis proceeds, the enzyme is inactivated, perhaps as the consequence of the formation of a polynucleotide structure which binds it irreversibly and thus prevents further degradation of the DNA, but does not affect ATP hydrolysis. A structure of the kind proposed by MacKay and Linn as an intermediate in the hydrolysis of duplex DNA (14) may serve as such an inhibitor. At low ATP concentrations, where hydrolysis of the duplex DNA proceeds to completion readily, there is a nearly simultaneous hydrolysis of both strands, thus preventing formation of the putative inhibitory structure.

It is possible that high concentrations of ATP limit the degradative capacity of the enzyme so as to ensure the generation of intermediates which may be essential for recombination. In view of the high intracellular concentration of ATP in *E. coli* (3×10^{-3} M) (15), the mode of attack of linear duplex DNA by the *rec*BC nuclease at these ATP concentrations may well reflect its true mode of action *in vivo*.

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REFERENCES

1. Goldmark, P. J., and Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860
2. Karu, A. E., MacKay, V., Goldmark, P. J., and Linn, S. (1973) *J. Biol. Chem.* **248**, 4874–4884
3. Oishi, M. (1969) *Proc. Natl. Acad. Sci. U. S. A.* **64**, 1292–1299
4. Nobrega, F. G., Rola, F. H., Pasetto-Nobrega, M., and Oishi, M. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69**, 15–19
5. Richardson, C. C. (1965) *Proc. Natl. Acad. Sci. U. S. A.* **54**, 158–165
6. Richardson, C. C. (1966) *J. Mol. Biol.* **15**, 49–61
7. Wu, R., Padmanabhan, R., and Bambara, R. (1974) Methods Enzymol **29E**, 231–253
8. Glynn, I. M., and Chapell, J. B. (1964) *Biochem. J.* **90**, 147
9. Schendel, P. F., and Wells, R. D. (1973) *J. Biol. Chem.* **248**, 8319–8321
10. Alberts, B. M., Amodio, F. J., Jenkins, M., Gutmann, E. D., and Ferris, F. C. (1968) *Cold Spring Harbor Symp. Quant. Biol.* **33**, 289–305
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
12. Wickner, W., Schekman, R., Geider, K., and Kornberg, A. (1973) *Proc. Natl. Acad. Sci. U. S. A.* **70**, 1764–1767
13. MacKay, V., and Linn, S. (1976) *J. Biol. Chem.* **251**, 3716–3719
14. MacKay, V., and Linn, S. (1974) *J. Biol. Chem.* **249**, 4286–4294
15. Lowry, O. H., Carter, J., Ward, J. B., and Glaser, L. (1971) *J. Biol. Chem.* **246**, 6511–6521