Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2215–2219, August 1973

Purification and Properties of the γ-Protein Specified by Bacteriophage λ: An Inhibitor of the Host RecBC Recombination Enzyme

(bacteriophage λ *gam* gene/RecBC DNase/exonuclease V)

YOSHIYUKI SAKAKI, ALEXANDER E. KARU, STUART LINN, AND HARRISON ECHOLS

Department of Molecular Biology and Department of Biochemistry, University of California, Berkeley, Calif. 94720

Communicated by Charles Yanofsky, May 9, 1973

ABSTRACT **Previous experiments have indicated that the *gam* gene of bacteriophage λ is responsible for an inhibition of the RecBC DNase—an enzyme that is essential for the major host pathway of genetic recombination. We report here experiments that define the inhibitor as the protein product of the *gam* gene ("γ-protein") and that characterize the inhibition reaction with highly purified preparations of γ-protein and RecBC DNase. Genetic characterization was performed with partially purified fractions prepared from cells infected with various λ mutants. An activity that inhibits RecBC DNase was absent in extracts prepared after infection by phage that carry nonsense or deletion mutations in the *gam* gene; this activity was highly thermolabile in an extract prepared after infection by phage that carry a temperature-sensitive mutation in the *gam* gene. For biochemical characterization, the γ-protein has been purified more than 800-fold. This highly purified preparation inhibited all of the known catalytic activities associated with the RecBC enzyme, but exhibited no detectable DNase or ATPase activities by itself. These findings are discussed in terms of their implications for regulation of genetic recombination and bacteriophage λ development.**

The product of the *gam* gene of phage λ participates in an interesting and complex variety of host–virus interactions, including: (*a*) the capacity of λ to grow (make a plaque) on a *recA*$^-$ *recB*$^+$ host [Fec phenotype (1)]; (*b*) the failure of λ to grow on a host lysogenic for phage P2 [Spi phenotype (1, 2)]; and (*c*) the inhibition of the host RecBC recombination pathway (3). A unifying hypothesis to explain these phenomena involves the postulate that phage λ converts *recB*$^+$ *recC*$^+$ cells to a phenotype characteristic of *recB*$^-$ or *recC*$^-$ mutants and that the *gam* gene of λ is required for this phenotypic conversion (see ref. 4 and *Discussion*).

Recent experiments have supported this postulate at a biochemical level: crude extracts from cells infected by *gam*$^+$ phage exhibit strongly diminished activity of the protein product of the *recB* and *recC* genes—the RecBC DNase—whereas extracts from cells infected by *gam*$^-$ phage show normal levels of RecBC DNase activity (5). Since this inhibitory effect could be demonstrated by mixing *gam*$^+$ and *gam*$^-$ extracts, the most likely explanation for the diminished activity of the RecBC DNase is an inhibition of enzyme activity, mediated directly or indirectly by the product of the *gam* gene ("γ-protein") (5, 6).

This report presents evidence that the inhibitor is the γ-protein itself and describes the purification of the γ-protein and its effects on purified RecBC DNase.

MATERIALS AND METHODS

Bacteriophage and Bacteria. The *Escherichia coli* strains used and their relevant genetic characteristics were: the Rec$^+$ Endonuclease I$^-$ strain JC6724 and its related *recB*21 *recC*22 double mutant JC6720; the Su$^-$ *recB*21 mutant JC4695 (3, 7). The λ phage mutations used were: *redX*314, a mutation in the structural gene for λ-exonuclease (8); *gam*210 and *gam*37, nonsense and temperature-sensitive mutations, respectively, in the *gam* gene (1); *bio*1, a deletion–addition mutation that has deleted most of the λ recombination region including the *gam* gene (1); *bio*72, a deletion–addition mutation that has deleted most of the λ recombination region, but not the *gam* gene (1). All phage except *bio*72*gam*37 carried the *cI*857 mutation, which renders the λ *cI* protein thermolabile and thus allows thermal induction of λ prophage (9).

DNA and Enzymes. E. coli [^{3}H]DNA (5500–7500 cpm/nmol) was prepared by the methods of Oishi (10), or Lehman (11). Phage T7 [^{3}H]DNA (17,000 cpm/nmol) was prepared according to Richardson (12); *E. coli* [^{3}H]DNA with interstrand crosslinks introduced by treatment with 4,5′,8–trimethylpsoralen was prepared as described (13).

The RecBC DNase used to monitor the purification of γ-protein ("Brij lysate preparation") was made as follows. A culture of *E. coli* JC6724 (6 to 8 × 10^8 cells per ml) was harvested, lysed with Brij, and fractionated with streptomycin sulfate and ammonium sulfate as described below for the purification of γ-protein. The ammonium sulfate fraction (1-ml) was layered on a gradient (11-ml) of 10–30% glycerol [in 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 0.1 M KCl] and centrifuged for 16 hr at 40,000 rpm in a Spinco SW41 rotor. Fractions about 65% of the way through the gradient contained the RecBC DNase. All studies with purified γ-protein (Fraction VII) used highly purified RecBC DNase [Fraction IX as described by Goldmark and Linn (14)] unless otherwise indicated.

Exonuclease I was the hydroxylapatite fraction, prepared and assayed according to Lehman and Nussbaum (15). Exonuclease II (homogeneous DNA polymerase) and the Sephadex G-100 fraction of Exonuclease III, prepared as described by Jovin *et al.* (16), were generously provided by Dr. D. Brutlag. *E. coli* B restriction endonuclease was the sucrose gradient fraction (17). Pancreatic RNase and RNase U_1, free of DNase, were provided by Dr. A. Blank.

Assay for γ-Protein measured its capacity to inhibit the activity of the RecBC enzyme. The preparation of γ-protein was "preincubated" with RecBC DNase, and then the substrate (usually double-stranded DNA) was added to measure remaining activity of the RecBC enzyme. For assay for exonuclease activity with double-stranded DNA and "Brij lysate preparation" of RecBC DNase, the following procedure was used. Preincubation mixtures contained: 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.5 mM ATP, and 0.2–0.6 units of "Brij lysate preparation" of RecBC

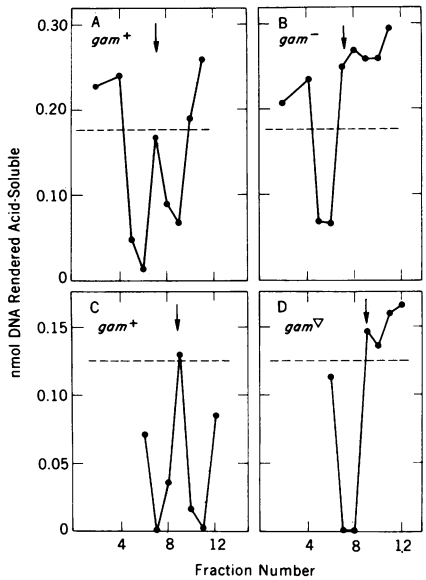

FIG. 1. Sedimentation properties of inhibitors of the RecBC DNase. The experiments of *A* and *B* used thermal induction of the *gam*$^+$ and *gam*$^-$ prophages in the lysogens JC4695(λ*cI*857 *redX*314) (*A*) and JC4695(λ*cI*857*redX*314*gam*210) (*B*). Cultures were grown at 30° and thermally induced by raising the temperature to 42° for 15 min. Extracts were prepared and purified through Fraction III, as described in *Methods*. The experiments of *C* and *D* used infection of JC4695 by the *gam*$^+$ and *gam*$^-$ deletion mutants λ*bio*72 (*C*) and λ*bio*1 (*D*). Cultures were grown at 37° and infected at a multiplicity of 4 phage per bacterium. After 15 min, extracts were prepared as for *A* and *B*. Sedimentation was done in a Spinco SW50.1 rotor at 4° in a 20–40% glycerol gradient [in 20 mM Tris·HCl (pH 8.0)–0.1 M KCl–1 mM EDTA–10 mM 2-mercaptoethanol]. For *A* and *B*, sedimentation was at 45,000 rpm for 28 hr; for *C* and *D*, sedimentation was at 49,000 rpm for 18 hr. Fractions were collected, and aliquots (5-μl) were assayed for inhibition of the RecBC DNase. Sedimentation was from right to left. *Arrows* denote the position of an internal hemoglobin marker ($s_{20,w}$ of 4.3, molecular weight of 64,000). (– – –), Uninhibited RecBC DNase activity added to the assay; (●——●), RecBC DNase activity in the presence of the gradient fractions.

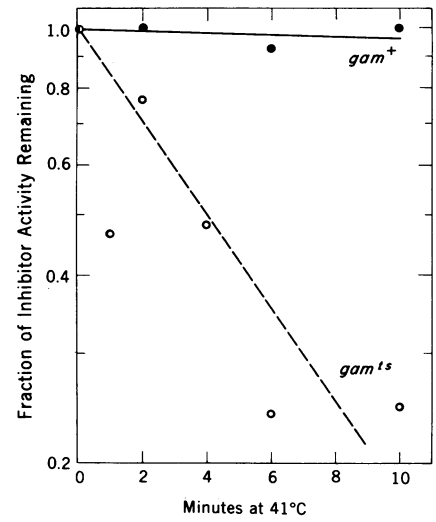

FIG. 2. Thermolability of Inhibitor II in an extract from a temperature-sensitive *gam* mutant. Cultures of JC4695 were grown at 28° and infected with λ*bio*72 (at a multiplicity of 4) or λ*bio*72*gamts*37 (at a multiplicity of 15). Cells were harvested 20 min after infection. Extracts were prepared and sedimented in glycerol gradients as described in Fig. 1. Samples of the inhibitory activity that sedimented more slowly than hemoglobin (Inhibitor II) were incubated at 41° for the times indicated, and then assayed (at 29°) for inhibition of RecBC DNase. (O– – –O), Inhibitor II activity from λ*bio*72*gamts*37; (●——●), Inhibitor II activity from λ*bio*72*gam*$^+$.

DNase in a total volume of 0.12 ml. The γ-protein was added at levels noted, and the mixture was incubated at 37° for 10 min. To this mixture was added 6 nmol of *E. coli* [^{3}H]DNA in 0.1 ml of 10 mM Tris·HCl (pH 8.0)–10 mM $MgCl_2$; incubation was continued for 20 min at 37°; 0.1 ml of bovine-serum albumin (4 mg/ml) was added, and the reaction was terminated by the addition of 0.04 ml of 50% trichloroacetic acid; the amount of nucleotide rendered acid-soluble was then determined (14). A reaction with ATP omitted was used to correct for DNA hydrolysis due to nucleases that contaminated less-purified fractions of γ-protein. One activity unit of RecBC DNase renders 1 nmol of DNA nucleotide acid-soluble in the assay described above. One activity unit of inhibitor eliminates one activity unit of "Brij lysate" RecBC DNase under these conditions; the assay is linear to 0.5 units.

In order to minimize loss of activity of purified RecBC enzyme due to preincubation at 37°, assays with purified RecBC DNase were conducted somewhat differently. Reactions (total volume 0.09 ml) contained: 10 mM Tris·HCl (pH 8.2), 10 mM $MgCl_2$, 1.1 mM dithiothreitol, 5 mM KPO_4 (pH 6.8), 0.55 mg/ml of acetylated bovine-serum albumin (14), 20% glycerol, about 1 unit (0.02-μg) of RecBC DNase, ATP and γ-protein as noted. After 10 min at 37°, 4 nmol of [^{3}H]DNA [in 0.06 ml of 10 mM Tris·HCl (pH 8.2)–10 mM $MgCl_2$] was added; incubation was continued for 30 min at 37°; 0.05 ml of bovine-serum albumin (10 mg/ml) was then added, and the reaction was terminated by addition of 0.25 ml of 7% trichloroacetic acid. For ATPase reactions, [γ-^{32}P]-ATP (500–1500 cpm/nmol) was used, and the ^{32}P rendered nonadsorbable to Norit was determined as described (14). The single-strand endonuclease assay was performed as described above, using circular fd [^{3}H]DNA and 0.7 units of exonuclease I (14).

Purification of γ-Protein. For the preparation used for most of this work, the endonuclease I-defective strain JC6720 (λ*cI*857*redX*314)* was grown at 30° with vigorous shaking in 7.5 liters of broth (per liter: 10 g of Difco Bacto Tryptone, 1 g of yeast extract, and 5 g of NaCl). At a density of 6 to 8 × 10^8 cells per ml, the λ prophage was thermally induced by raising the temperature of the culture to 42° for 15 min; the culture was then rapidly chilled to 4°. Cells were harvested by centrifugation, washed with 0.85% NaCl, and suspended in 50 ml of 10 mM Tris·HCl (pH 8.2), containing 25% sucrose. The cells were disrupted with Brij-58 and lysozyme, as described by Unger and Clark (5), and debris was removed by centrifugation at 27,000 × *g* for 15 min (Fraction I, Table 1).

To Fraction I (120-ml) was added, with stirring, 30 ml of 0.2 M EDTA (pH 7.0) and 30 ml of 5% streptomycin sulfate

* If purified RecBC enzyme is used, it is important that the host be *endI*$^-$ to avoid interference in the assay by endonuclease I present in inhibitor Fractions I through IV.

(freshly prepared, and adjusted to pH 8.0 with Tris). After 20 min at 4° the precipitate was removed by centrifugation at 17,000 × *g* for 10 min and discarded (Fraction II). To Fraction II (183-ml), 31 g of solid ammonium sulfate was slowly added with stirring. After 10 min, the precipitate and the Brij-58 (which floats on the supernatant solution) were removed by centrifugation at 12,000 × *g* for 10 min. To the supernatant, 42 g of ammonium sulfate was added. After 30 min the precipitate was collected by centrifugation, dissolved in 1 ml of Buffer A [20 mM Tris·HCl (pH 8.2)–1 mM EDTA–0.1 M KCl–10 mM 2-mercaptoethanol–10% glycerol], and cleared by centrifugation (Fraction III).

Portions (6-ml) of Fraction III were applied to a 2.5 × 85-cm column of Sephadex G-100 equilibrated with Buffer A. Upon elution with Buffer A, inhibitory activity appeared between 277 and 308 ml (Fraction IV). Fraction IV was applied to a 1 × 8-cm column of DEAE-cellulose (Whatman DE-52), equilibrated with Buffer A. The column was washed with 20 ml of Buffer A, and the γ-protein was eluted with a linear gradient (120 ml total volume) from 0.1–0.4 M KCl in Buffer A. Inhibitory activity eluted between 0.24 and 0.27 M KCl. Fractions with more than 50 units/ml were pooled and dialyzed against Buffer B [20 mM KPO_4 (pH 6.7)–10 mM 2-mercaptoethanol–10% glycerol] (Fraction V). Fraction V (23 ml) was applied to a 1 × 5-cm column of phosphocellulose (Whatman P-11) equilibrated with Buffer B, and the column was washed with 20 ml of Buffer B. The inhibitory activity was collected in the unadsorbed material (Fraction VI).

Fraction VI was applied to a 1 × 1-cm column of hydroxylapatite (Bio-Gel HT) in Buffer B. The column was washed with 3 ml of Buffer B, and γ-protein was eluted with a linear gradient (20 ml total volume) from 0.02–0.4 M KPO_4 (pH 6.8) in Buffer B. Inhibitory activity eluted between 0.18 and 0.27 M KPO_4. Fractions with more than 32 units/ml were pooled (3.5 ml) and dialyzed against 10 mM KPO_4 (pH 6.8)–10 mM 2-mercaptoethanol–10% glycerol; dialysis was continued against the same buffer with glycerol increased to 50%. During this dialysis the γ-protein fraction was concentrated to a final volume of 1.1 ml (Fraction VII). Portions of Fraction VII (0.15-ml) were subjected to electrophoresis in 5% polyacrylamide gels, essentially as described by Davis (18). Although there was too little protein in the gel to be detected by staining, inhibitory activity was eluted from a region with a mobility of 0.71 relative to the bromphenol-blue tracking dye. This gel fraction and Fraction VII showed identical inhibitory activity toward the RecBC enzyme. Fraction VII was stored at −20° for 2 months with no appreciable loss of activity. Fractions V and VI were unstable, and lost roughly 80% of their inhibitory activity within 1 week at 4°.

A large-scale preparation of γ-protein has also been made from a 200-liter culture of JC6720(λ*x*13cI857). The *x* mutation was chosen because its *cro*⁻ phenotype provides for overproduction of proteins of the recombination region (19, 20). The large preparation was essentially a scaled-up version of that described above, except that sonication was used to produce lysis instead of lysozyme and Brij.

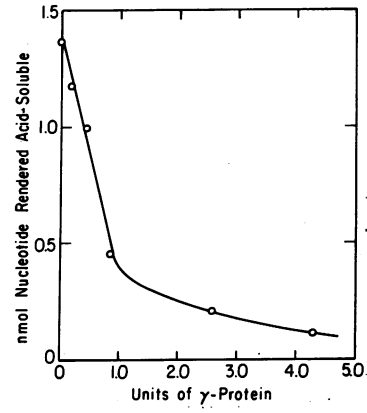

FIG. 3. Inhibition of double-strand exonuclease activity of RecBC DNase by purified γ-protein. The activity of purified RecBC DNase (1.4 units) was assayed after preincubation with various quantities of γ-protein as described in *Methods*. The ATP concentration was 0.28 mM in the preincubation and 0.17 mM in the assay for DNase activity.

TABLE 1. *Purification of γ-protein*

Fraction	Volume (ml)	Protein (mg/ml)	Inhibitor units*	Specific activity (units/mg of protein)
I Brij lysate	120	4.6	34,200	62
II Streptomycin	183	1.9	48,900	140
III Ammonium sulfate	11	24	20,800	78
IV Sephadex G-100	65	0.17	11,500	1,040
V DEAE–cellulose	24	~0.003	2,220	~32,000
VI Phosphocellulose	17	~0.003	455†	>9,000†
VII Hydroxylapatite	3.5	≲0.001	175	≳50,000

The assays for inhibitory activity were conducted as described in *Methods*, with the "Brij-lysate preparation" of RecBC DNase. Protein was determined by the method of Lowry *et al.* (28), except that Fraction VII did not contain a detectable level of protein, either by the Lowry method, or by absorbance at 215, 225, or 280 nm.

* Because of the presence of nonspecific inhibitory material in Fractions I to III, the activity units reported for these fractions do not specifically measure inhibition by γ-protein.

† This fraction is unstable, and a substantial loss of activity had occurred before assay.

TABLE 2. *Effect of γ-protein on activities catalyzed by the RecBC enzyme*

RecBC activity	DNA present	ATP conc. (mM)	% Inhibition
Exonuclease	Double-strand linear	0.17	38
		3.5	44
Exonuclease	Single-strand linear	0.17	43
		3.5	49
Endonuclease	Single-strand circular	0.0	31
		0.33	58
ATPase	Double-strand linear	0.17	37
ATPase	Double-strand crosslinked	0.17	44

Reactions were conducted as described in *Methods*, with 4.3 nmol of DNA, ATP as indicated, 0.2 unit of γ-protein and sufficient RecBC enzyme to degrade 1 nmol of DNA in the absence of γ-protein. The level of ATP noted above was that present in the assay of RecBC activity; preincubation levels were 1.7-fold higher. All assays used *E. coli* DNA (native, denatured, or crosslinked), except for the endonuclease assay, which used circular DNA from phage fd (14).

RESULTS

Evidence That the γ-Protein is an Inhibitor of RecBC DNase. As a first step in analysis of the apparent inhibition of RecBC DNase mediated by phage λ, we sought inhibitory activity in crude extracts of a thermally induced lysogen of the *recB*$^-$ strain JC4695(λcI857 *redX*314). The *recB*$^-$ mutant was chosen in order to study only effects on added RecBC enzyme, and the *redX*$^-$ mutant of λ was used to avoid complications by λ-exonuclease activity. Initial experiments revealed inhibitory activity in extracts from cells in which λ was induced to lytic growth, but also showed some inhibitory activity in extracts from uninduced cells.

In an effort to clarify the nature of the inhibitory activities, we sedimented crude preparations through glycerol gradients. Two peaks of inhibitory activity were found: one that sedimented more rapidly than hemoglobin (Inhibitor I) and one that sedimented less rapidly (Inhibitor II) (Fig. 1*A*). Inhibitor I has been found in every extract that we have tested (including extracts from *recA*$^-$ cells), whether or not a λ phage was present. Inhibitor II has been found only in cells in which the λ *gam* gene is expressed; this activity is absent if nonsense or deletion mutants of the *gam* gene are used (Fig. 1*B* and *D*).

To investigate whether Inhibitor II is actually the product of the *gam* gene, we used glycerol gradient sedimentation to prepare this inhibitor from cells infected by λ phage that carried a temperature-sensitive mutation in the *gam* gene. Inhibitor II was much more thermolabile in such extracts than in extracts made after *gam*$^+$ infection (Fig. 2).

From the results of Figs. 1 and 2, we conclude that the product of the *gam* gene of phage λ ("γ-protein") is probably itself an inhibitor of the RecBC DNase.

General Properties of γ-Protein. The Inhibitor II activity (which we will call γ-protein) was purified as described in *Methods*. We believe the inhibitor is a protein because: (*a*) activity is abolished by a nonsense mutation in the *gam* gene and is highly thermolabile for a temperature-sensitive mutation in the *gam* gene; (*b*) the chromatographic and sedimentation characteristics are those expected of a protein; (*c*) activity is retained after exposure to pancreatic RNase and RNase U_1; and (*d*) activity is lost after exposure to 95° for 5 min. From its elution properties on Sephadex G-100 and its sedimentation characteristics (see Fig. 1), we estimate a molecular weight for γ-protein of about 30,000.

Purified γ-protein is not itself a nuclease or ATPase. No detectable exonuclease or endonuclease activity was found for either single-stranded or double-stranded DNA (at pH 7.0 or 9.0), regardless of whether ATP was present. (The assay for double-stranded endonuclease activity was capacity to cleave or nick a circular DNA substrate, as judged by sedimentation analysis.) No detectable hydrolysis of ATP was observed, in the presence or absence of RecBC enzyme or DNA.

Inhibition of Double-Strand Exonuclease Activity of RecBC DNase. The γ-protein was purified on the basis of its capacity to inhibit the ATP-dependent, double-strand exonuclease activity of the RecBC DNase. We shall first summarize the properties of this reaction and then consider the effect of γ-protein on the other activities of the RecBC enzyme.

In the presence of inhibitor, the nuclease reaction proceeded at a constant, but reduced rate throughout the time of incubation. The degree of reduction was proportional to the amount of γ-protein present up to about 0.9 unit, above which the inhibition became nonlinear (Fig. 3). The maximum extent of inhibition has varied from 65–90% with different preparations of γ-protein. We think that the most likely explanation for this variability is the presence of inactive γ-protein that can compete with active material.

Inhibition of RecBC activity was optimal if the enzyme and γ-protein were first incubated with ATP before DNA was added to initiate the exonuclease reaction. The extent of inhibition increased with the time of preincubation, and became maximal after 10 min; this pattern was independent of the amount of γ-protein present. If preincubation was omitted, inhibition was reduced by about 50%. The preincubation was equally efficient at temperatures from 15°–37° and ATP concentrations from 28 μM–5.8 mM.

Inhibition of Other Activities of RecBC DNase. Besides the double-strand exonuclease activity, the RecBC enzyme possesses three other activities: single-strand exonuclease, single-strand endonuclease, and DNA-dependent ATPase (14). An obvious question is whether all activities of the RecBC enzyme are inhibited. Table 2 gives the results of experiments done to investigate this point. The γ-protein inhibited all four activities to about the same extent. The degree of inhibition was largely independent of ATP concentration, in contrast to the large effects of ATP concentration on the activities of the RecBC enzyme (14). Substantial inhibition was found even for the ATP-independent, single-strand endonuclease activity (Table 2, line 5). Thus the γ-protein does not exert its effect solely on the interaction of RecBC enzyme and ATP. On the other hand, inhibition of the DNA-dependent ATPase activity was also found with crosslinked DNA, which is not a substrate for the nucleolytic reaction (13) (Table 2, line 8). Therefore, the interaction with ATP can be affected in the absence of DNA degradation.

In addition to production of small fragments from double-stranded DNA, the RecBC enzyme releases large, sedimentable products in reactions in which the ATP level is high (21). We have examined the effect of γ-protein on production of these large fragments from DNA of phage T7; as judged by sedimentation analysis, the formation of both large and small fragments was reduced to the same extent by γ-protein. Thus, γ-protein does not appear to produce an obvious qualitative alteration of the products of the nucleolytic reaction.

To investigate the specificity of the inhibitory activity of γ-protein, we have studied in some detail the effect of γ-protein on three other nucleases: exonuclease II, the restriction enzyme from *E. coli* B, and exonuclease III. The DNase activity of exonuclease II and the DNA-dependent ATPase activity of the restriction enzyme were not significantly affected by γ-protein (inhibition <4%). The activity of exonuclease III was diminished by about 20% at levels of γ-protein that were saturating for the RecBC enzyme. The large excess of bovine-serum albumin present in the reaction mixture had no effect on inhibition by γ-protein.

From the results of the enzyme studies described above, we conclude that the γ-protein is an effective inhibitor of all activities of the RecBC enzyme, but is not a general inhibitor of all exonucleases or DNA-dependent ATPases. The small inhibition of exonuclease III is an interesting effect that requires further study.

DISCUSSION

Our results provide a direct biochemical verification of an inference derived from various previous studies: that the

gam gene of phage λ specifies an inhibitor of the RecBC DNase (3–5, 22, 23). Two important questions remain incompletely answered: What is the nature of the interaction between γ-protein and the RecBC enzyme, and what is the importance of this interaction for phage λ development and recombination?

In vitro the γ-protein inhibits all the diverse activities of the RecBC DNase, but is not itself a nuclease and does not have detectable ATPase activity. Thus, the most likely mechanism for the inhibition appears to be a regulatory interaction with the RecBC enzyme. The enhancement of inhibitory activity by ATP is consistent with various possibilities, but may eventually provide a clue as to the mechanism of the γ-mediated inhibition and possibly other regulatory aspects of the RecBC enzyme (e.g., phosphorylation reactions or ATP-induced conformational changes).

To what extent does the inhibitory activity of the γ-protein provide an explanation for the known effects of γ-protein *in vivo*? This activity provides a direct explanation for one phenomenon—the capacity of phage λ to inhibit the major host recombination pathway, which is dependent on the RecBC enzyme (3). This regulatory action at the protein level may be representative of a wider class of similar regulatory effects in recombination. For example, the RecA protein might control the activity of the RecBC enzyme, and pathways of host recombination alternative to the RecBC pathway might also be regulated at the level of enzyme activity (see ref. 24).

The inhibitory activity of γ-protein also provides a plausible explanation for the more complex Fec phenotype—the inability of phage λ to grow in a *recA*$^-$ host if the phage carries mutations in one of the λ general recombination genes (*red*) *and* in the *gam* gene (1). This effect can be understood if concatemeric λ DNA is required for maturation (22, 25) and if concatemer formation can occur through either a general recombination pathway (phage Red or bacterial Rec) or a replicative pathway antagonized by the RecBC DNase (22). In this context, it is interesting to note that phage T4 also inhibits host RecBC DNase (26) and phage P22 inhibits a possibly analogous enzyme in its host *Salmonella* (27).

The inhibitory activity of γ-protein provides a formal, but not biochemical explanation for part of the even more complex Spi phenotype—the ability of phage λ to grow effectively in a P2 lysogen only if the λ phage carries mutations in the *red* and *gam* genes and in another gene of the recombination region termed *del* (1, 2). A phage P2 gene, termed *old*, appears to effect an inhibition of protein and RNA synthesis in *recB*$^-$ or *recC*$^-$ cells. Therefore, the presence of γ-protein will contribute to the demise of phage λ because the inhibition of the RecBC enzyme makes normal cells into a phenotypic analog of *recB*$^-$ or *recC*$^-$ (4).

One effect of *gam*$^-$ mutation that is not explained by the inhibitory activity of γ-protein is the approximately 2-fold reduction in recombination by the phage Red pathway found for *gam*$^-$ mutants of λ in *recA*$^-$ hosts (1). This result might indicate a direct, but minor participation by γ-protein in the Red pathway (1) or might result from the deleterious effects of an uncontrolled RecBC DNase on recombinational intermediates (3). This point may be clarified by a study of possible interaction between γ-protein and the proteins of the Red pathway: λ-exonuclease and β-protein.

We thank Barry Egan, Lynn Enquist, Linda Pilarski, and Ann Skalka for the communication of unpublished information; John Clark and Richard Unger for advice; James Zissler for phage strains; and Vivian Mackay for various fractions of RecBC enzyme. This investigation was supported in part by Public Health Service Research Grants GM 17078 and GM 19020 from the National Institute of General Medical Sciences, and Contract no. AT(04-3)-34 from the U.S. Atomic Energy Commission. A.E.K. is a Postdoctoral Fellow (Grant CA 52887) of the National Cancer Institute.

1. Zissler, J., Signer, E. & Schaefer, F.(1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 455–468.
2. Lindahl, G., Sironi, G., Bialy, H. & Calender, R. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 587–594.
3. Unger, R. C., Echols, H. & Clark, A. J. (1972) *J. Mol. Biol.* **70**, 531–537.
4. Sironi, G., Bialy, H., Lozeron, H. A. & Calendar, R. (1971) *Virology* **46**, 387–396.
5. Unger, R. C. & Clark, A. J. (1972) *J. Mol. Biol.* **70**, 539–548.
6. Unger, R. C. (1972) Ph.D. thesis, University of California, Berkeley.
7. Barbour, S. D., Nagaishi, H., Templin, A. & Clark, A. J. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 128–135.
8. Shulman, M. J., Hallick, L. M., Echols, H. & Signer, E. R. (1970) *J. Mol. Biol.* **52**, 501–520.
9. Sussman, R. &. Jacob, F. (1962) *C. R. H. Acad. Sci.* **254**, 1517–1519.
10. Oishi, M. (1969) *Proc. Nat. Acad. Sci. USA* **64**, 1292–1299.
11. Lehman, I. R. (1960) *J. Biol. Chem.* **235**, 1479–1487.
12. Richardson. C. C. (1966) *J. Mol. Biol.* **15**, 49–61.
13. Karu, A. E. & Linn, S. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2855–2859.
14. Goldmark, P. J. & Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860.
15. Lehman, I. R. & Nussbaum, A. L. (1964) *J. Biol. Chem.* **239**, 2628–2636.
16. Jovin, T. M., Englund, P. T. & Bertsch, L. L. (1969) *J. Biol. Chem.* **244**, 2996–3008.
17. Eskin, B. & Linn, S. (1972) *J. Biol. Chem.* **247**, 6183–6191.
18. Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427.
19. Radding, C. M. (1964) *Proc. Nat. Acad. Sci. USA* **52**, 965–973.
20. Pero, J. (1970) *Virology* **40**, 65–71.
21. Karu, A. E., Mackay, V., Goldmark, P. J. & Linn, S. (1973) *J. Biol. Chem.*, in press.
22. Enquist, L. W. & Skalka, A. (1973) *J. Mol. Biol.* **75**, 185–212.
23. Pilarski, L. M. & Egan, J. B. (1973) *J. Mol. Biol.* **76**, 257–266.
24. Clark, A. J. (1971) *Annu. Rev. Microbiol.* **25**, 437–464.
25. Stahl, F. W., McMilin, K. D., Stahl, M. M., Malone, R. E., Nozu, Y. & Russo, V. E. A. (1972) *J. Mol. Biol.* **68**, 57–67.
26. Tanner, D. & Oishi, M. (1971) *Biochim. Biophys. Acta* **228**, 767–769.
27. Israel, V., Woodworth-Gutai, M. & Levine, M. (1972) *J. Virol.* **9**, 752–757.
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.