X-Ray Inactivation of the *Escherichia coli* Deoxyribonucleic Acid Dependent Ribonucleic Acid Polymerase in Aqueous Solution. I. Studies on Binding and Substrate Concentration Dependence in the Inactivation of Enzymatic Activity†

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ABSTRACT: The X-ray inactivation of enzymatic activity of the DNA-dependent RNA polymerase isolated from three varying radiosensitive strains of *Escherichia coli* was examined using a variety of homopolymer templates and a natural double-stranded DNA. The radioresponse of enzymatic activity of the polymerase was independent of radiosensitivity of the cell from which it was extracted. Although the inactivation profiles obeyed single-hit exponential kinetics, the resultant D_{37} values obtained varied according to the template molecule used in the reaction assay. This variability in dose–response of the enzyme could not be attributed to binding of irradiated enzyme to the template molecule; the D_{37} values for inactivation of binding of irradiated polymerase to a poly(U) or poly(dT) template were four to eight times greater than corresponding D_{37} values for inactivation of enzymatic activity of the polymerase using these two template molecules. When irradiated enzyme was assayed with homoribopolymer templates, there existed a substrate dependence in inactivation process (*i.e.*, the D_{37} values for inactivation of enzymatic activity of the RNA polymerase changed as the substrate concentration in the reaction mixture was varied). With homodeoxyribopolymer templates, a substrate independence in inactivation curves was observed. Variation in average size of the template molecule did not influence inactivation of enzymatic activity of the X-irradiated RNA polymerase. Results from this paper, along with supporting data in the following paper, strongly suggest that the initiation step in the catalytic process of RNA polymerization is the radiosensitive target.

The DNA-dependent RNA polymerase has been isolated from several genera of bacteria and shown to catalyze, in the presence of nucleoside triphosphates and a divalent metal ion, the polymerization of complementary ribonucleic acid polymers using a variety of synthetic ribo- and deoxyribonucleic acid templates and natural DNAs (Chamberlin and Berg, 1962; Krakow and Ochoa, 1963; Fox *et al.*, 1964; Niyogi and Stevens, 1965a,b). The basic mechanism of RNA polymerization involves several discrete steps consisting of (1) binding of the polymerase to the template molecule; (2) initiation or association of the first nucleoside triphosphate with the enzyme–template complex; (3) elongation or addition of nucleoside triphosphates to the nascent RNA polymer with release of pyrophosphate; and (4) termination or release of the product RNA polymer from the enzyme–template complex (Anthony *et al.*, 1966; Walter *et al.*, 1967). RNA polymerase is a complex enzyme consisting of several polypeptide subunits; the holoenzyme is composed of the following subunits: $\alpha_2\beta\beta'\sigma(\omega)$ (Zillig *et al.*, 1970; Burgess and Travers, 1971). It has been shown that various chemical and antibiotic treatments of RNA polymerase affect RNA polymerization at a particular step(s) and that specific subunits of RNA polymerase are the targets of such treatments which lead to an abortive reaction (Ishihama and Hurwitz, 1969; Heil and Zillig, 1970; Zillig *et al.*, 1970; Smith *et al.*, 1971; Ishihama, 1972).

Another agent which can affect RNA synthesis is ionizing radiation. From the studies of Pollard (1970) and coworkers it has been shown that controlled transcription *in vivo* in the bacterium *Escherichia coli* is sensitive to ionizing radiation; furthermore, it was possible to correlate, from critical target analysis, the radiosensitive structure responsible for utilization of uracil, an RNA precursor, with a structure similar in size to the RNA polymerase. Sümegi and colleagues (1971, 1972) have shown recently that RNA polymerase isolated from *E. coli* is inactivated as an exponential function of dose (X-ray) when the enzyme was assayed using calf thymus DNA. When T4 DNA was used as the template molecule, complex inactivation kinetics were seen. This complex inactivation process seen in the latter case was attributed, in part, to inactivation of the σ subunit which contains highly reactive sulfhydryl groups and is necessary for proper initiation on some template molecules. It was also found that molecular size distribution of the RNA product polymerized by X-irradiated RNA polymerase was significantly reduced; this reduction was attributed to premature release of the polymerase from the synthesizing complex (Sümegi *et al.*, 1972).

The present study concerns the effects of ionizing radiation on the overall catalytic process of *in vitro* RNA polymerization. RNA polymerase isolated from three strains of *E. coli* of varying radiosensitivity was X-irradiated in air-saturated solutions at 0°, and the rates of polymerization were assayed using a variety of synthetic polymers and natural DNA templates. Furthermore, as described in this and the following paper (Strniste *et al.*, 1973), we examined separately the various discrete steps in the RNA polymerization process (binding, initiation, and elongation) in order to establish which step(s) could account for radiosensitivity of the enzyme. The following paper is concerned also with the possible influence of radiation on fidelity of RNA transcription. Results of these studies indicate that radiation can perturb the mechanisms

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of DNA-directed RNA synthesis and suggest that such perturbations could contribute to both sublethal and lethal effects experienced by cells exposed to ionizing radiation.

Experimental Procedures

Isolation of Polymerase. The DNA-dependent RNA polymerase containing σ factor was isolated from three strains of *E. coli*: B/r, B_{s-1}, and K12. From 200 g of cells of each strain, the polymerase was isolated according to the procedure of Burgess (1969a) utilizing, in the final purification steps, passage of the protein through two agarose columns: a Bio-Gel A-5m and a Bio-Gel A1.5m (Bio-Rad Laboratories) at low and high salt concentrations, respectively. From the extinction coefficient of purified RNA polymerase, $\epsilon^{1\%}_{280\,nm} = 6.5$ (Richardson, 1966), and the polymerizing activity of the enzyme using salmon sperm DNA as a template, the final specific activity of the three individual preparations was approximately 300 units/mg of protein (one unit of enzyme incorporates 1 nmol of AMP in a 10-min incubation under conditions as described below). Electrophoresis of all three preparations was conducted on 0.1% sodium dodecyl sulfate polyacrylamide gels as described elsewhere (Shapiro *et al.*, 1967; Burgess, 1969b); the results indicated homogeneity and purity for all enzyme preparations. Isolated polymerase was stored at 8 mg/ml in 0.01 M Tris (Schwarz/Mann, Ultra Pure grade) (pH 7.7), 0.01 M $MgCl_2$, 0.1 M KCl, 0.01 mM EDTA, 0.1 mM dithiothreitol, and 10% glycerol (this mixture is defined as storage buffer) at −179°; no loss in activity of the enzymes was observed over a 9-month storage interval.

Materials. All chemicals were of analytical grade unless otherwise specified. Radioactive NTPs (^3H and ^{14}C) were obtained from Schwarz/Mann, and unlabeled NTPs were purchased from P-L Biochemicals, Inc.

Synthetic Polymers. Poly(U), poly(A), and [^3H]poly(U) were purchased from Miles Laboratories, Inc. Poly(dT), poly(dA), poly(dU), and [^3H]poly(dT) were synthesized using oligonucleotide initiators, deoxynucleoside 5′-triphosphates, and calf thymus terminal deoxyribonucleotidyltransferase (Hayes *et al.*, 1966). From the elution profiles obtained from a calibrated Bio-Gel A50m column, homopolymers synthesized in this laboratory and mentioned in this and the following paper had the following average monomer unit lengths: poly(dA), 446; poly(dT), 39, 398, 404, 502, and 1210; poly(dU), 170; and [^3H]poly(dT), 193. The poly(U) purchased from Miles Laboratories, Inc., had average monomer unit lengths of 227 and 421 (two independent samples assayed).

X-Irradiation. To prepare the RNA polymerase for irradiation, samples of the enzyme were diluted twofold with 0.01 M Tris–0.15 M KCl (pH 7.9), and dialyzed overnight at 4° against three changes of 500-fold volumes of the same buffer. The samples then were further diluted with the Tris-KCl buffer so that the final protein concentration was 0.6–0.7 mg/ml. Recovery of enzymatic activity after extensive dialysis was greater than 95%.

Irradiation was performed using a General Electric Maxitron X-ray source; the parameters of irradiation were 250 kV, 30 mA with a 0.4-mm Sn, 0.25-mm Cu, and 1.0-mm Al filter. The dose rate averaged 420 rads/min. Samples between 200 and 400 μl of dialyzed RNA polymerase were placed in 0.5-dram glass vials, arranged on an isodose line, set in ice water, and irradiated for specific times. Immediately after irradiation, the samples were diluted twofold with double storage buffer with 10 mg/ml of bovine serum albumin and stored at 0°. Inactivation curves of the irradiated RNA polymerase did not change over a 2-week period when the enzyme was stored under the described conditions.

Polymerization Assays. Reactions assayed using the double-stranded salmon sperm DNA template contained per 250 μl: 10 μmol of Hepes[1] buffer (pH 7.9) (Calbiochem, A grade); 1.0 μmol of $MgCl_2$; 0.25 μmol of $MnCl_2$; 0.15 μmol each of ATP, GTP, UTP, and CTP (ATP labeled with either ^{14}C or ^3H at 495 or 1110 cpm per nmol, respectively); 40 μmol of KCl; 2.5 μmol of 2-ME; 110 μg of salmon sperm DNA (Worthington Biochemical Corp.), and RNA polymerase. Assay mixtures were incubated at 37° for desired times, spotted on glass fiber disks (Whatman GF-82), and soaked in 5% CCl_3COOH–0.01 M $Na_4P_2O_7$ for 10 min at 0°. The filters were then washed under suction with 25 ml of 2% CCl_3COOH–0.01 M $Na_4P_2O_7$ (0°), rinsed with 95% ethanol, and dried. The discs were placed in scintillation vials filled with 15 ml of toluene base scintillation cocktail and counted in a Packard Tri-Carb scintillation spectrometer (Model 3003).

Reactions assayed using the homoribopolymers poly(U) and poly(A) contained per 225 μl (unless stated differently elsewhere): 10 μmol of Hepes buffer (pH 7.9), 0.5 μmol of $MnCl_2$, 2.5 μmol of 2-ME, 120 nmol of homoribopolymer,[2] and 375 nmol of NTP (450 cpm/nmol of [^{14}C]ATP, 410 cpm/nmol of [^{14}C]UTP) plus RNA polymerase.

Reactions assayed using the homodeoxyribopolymers (poly(dT), poly(dU), and poly(dA)) contained per 225 μl (unless stated differently elsewhere): 10 μmol of Hepes buffer (pH 7.9), 2.0 μmol of $MgCl_2$, 2.5 μmol of 2-ME, 120 nmol of homodeoxyribopolymer, and 375 nmol of NTP (450 cpm/nmol of [^{14}C]ATP and 410 cpm/nmol of [^{14}C]UTP) plus RNA polymerase. The exact procedures of incubation, precipitation, and counting reaction mixtures, as noted above, were followed in the reactions assayed using the homopolymer templates.

Binding Assay. The binding of RNA polymerase to synthetic homopolymers was similar to procedures of Jones and Berg (1966) and Ishihama and Hurwitz (1969). The binding mixture contained per 250 μl: 12.5 μmol of Hepes buffer (pH 7.9), 1.0 μmol of $MnCl_2$, 1.0 μmol of 2-ME, 2.6 nmol of template, and varying amounts of RNA polymerase. The binding wash mixture consisted of 0.01 M Tris buffer and 0.05 M KCl (pH 7.6).

In the binding procedure, [^3H]poly(U) or [^3H]poly(dT) (2.6 nmol each, 1.1×10^4 cpm/nmol of [^3H]poly(U) and 6.7×10^3 cpm/nmol of [^3H]poly(dT)) was mixed with varying amounts of RNA polymerase in the binding mixture at 37° and incubated for 5 min. The sample was then diluted with 5 ml of chilled (0°) binding wash mixture and kept at 0° for more than 5 min. In the original noted procedure (Jones and Berg, 1966), Millipore filters were used to collect the enzyme–polymer complex; however, due to extensive retention of single-stranded polymers on Millipore filters at low salt concentrations, we substituted these filters with glass fiber disks (Whatman GF-82) which trap enzyme–polymer complexes yet allow free passage of unbound single-stranded polymers.[3] The glass fiber filters were presoaked at least 10 min in the binding wash mixture before use. Samples were applied to filters with gentle suction (20 mm) and rinsed with 50 ml of binding wash

[1] Abbreviations used are: NTP, nucleoside triphosphate; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; 2-ME, 2-mercaptoethanol.

[2] Quantities of synthetic homopolymers are always expressed in terms of monomer units.

[3] Details to be published elsewhere.

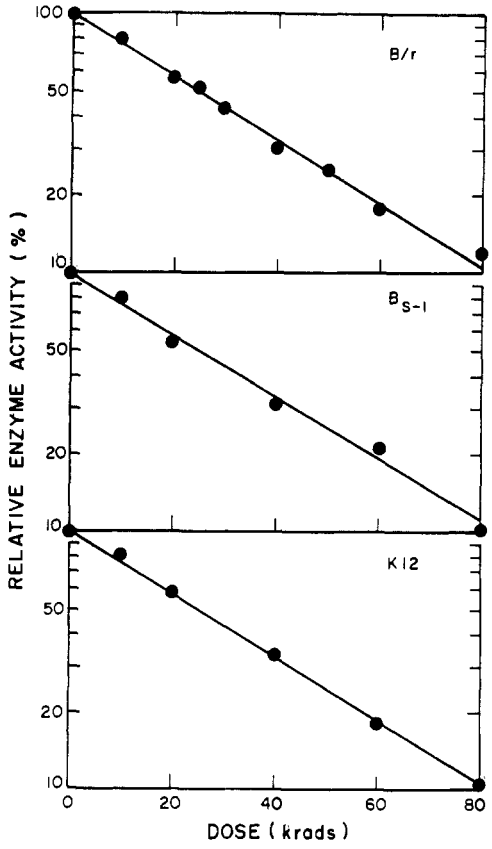

FIGURE 1: Inactivation of the enzymatic activity of *E. coli* B/r, B_{s-1}, and K12 RNA polymerases by X-irradiation when assayed with salmon sperm DNA. Irradiation and assay conditions were as described in the Experimental Procedures. The nonirradiated control enzyme samples (7.5–8.0 μg) incorporated 2.3 nmol of [^{14}C]-AMP in 10-min incubation at 37°.

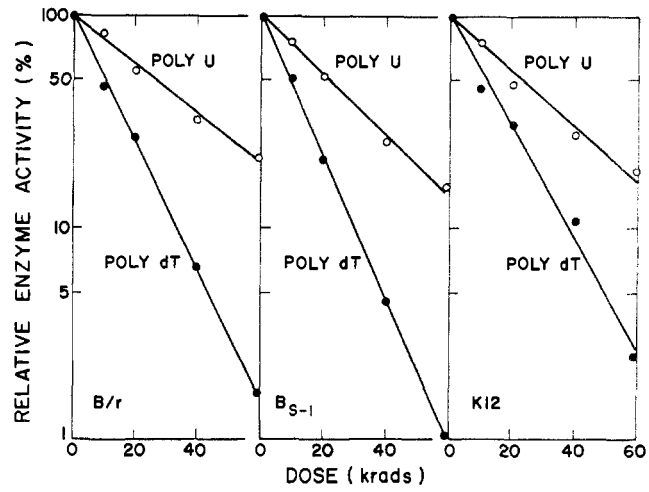

FIGURE 2: Inactivation of the enzymatic activity of *E. coli* B/r, B_{s-1}, and K12 RNA polymerases by X-irradiation when assayed with either poly(U) or poly(dT). Assays contained substrate at 375 nmol of [^{14}C]ATP (4.5×10^8 cpm/μmol) per reaction. The nonirradiated RNA polymerase samples (7.5–8.0 μg) incorporated 4 nmol of [^{14}C]AMP in 20 min using a poly(U) template plus Mn^{2+} or 18 nmol of [^{14}C]AMP in 10 min using a poly(dT) template plus Mg^{2+}.

mixture (0°), followed by 5 ml of chilled 50:50 (v/v) ether–ethanol mixture. The filters were dried, placed into scintillation vials with a toluene-base cocktail, and counted.

Results

Inactivation of RNA Polymerase with X-Irradiation. ENZYMATIC ACTIVITY WITH SALMON SPERM DNA TEMPLATE. Survival curves for X-irradiated RNA polymerase, as expressed in per cent remaining activity as a function of dose, are shown in Figure 1. It is apparent that, under the irradiation conditions employed (see Experimental Procedures), X-ray inactivation of *E. coli* B/r, B_{s-1}, and K12 RNA polymerases is similar. The D_{37} values (dose required to reduce enzymatic activity to 37% of the control, nonirradiated value) from the single-hit exponential curves are 35–36 krads, from which the yield of inactivation or *G* value (number of enzyme molecules inactivated per 100 eV absorbed) is calculated to be 0.046–0.047. It should be noted that each data point in Figure 1 represents between three and nine independent experiments, from which all data were averaged and normalized; the largest standard deviation calculated [σ (%) ±] equaled 4.

ENZYMATIC ACTIVITY WITH SYNTHETIC SINGLE-STRANDED HOMOPOLYMER TEMPLATES. Using a variety of homoribo- and homodeoxyribopolymers as templates, inactivation of enzymatic activity of the RNA polymerase by X-irradiation was examined. The effect of X-irradiation upon the ability of the B/r, B_{s-1}, and K12 RNA polymerases to catalyze poly(A) using a poly(U) or poly(dT) template is shown in Figure 2. The inactivation curves obey single-hit exponential kinetics; however, the D_{37} values for all three RNA polymerases assayed with poly(U) averaged 34 krads, in contrast to an average D_{37} of 16 krads for the three enzymes when assayed with poly(dT). It should be emphasized that this greater than twofold decrease in D_{37} values, when the enzymatic activity of the RNA polymerase using a poly(dT) template is compared to the activity of the enzyme when a poly(U) template is used, is observed at high substrate concentrations in the reaction mixture. In the above experiment, there were 375 nmol of ATP/reaction vessel. In the latter part of this paper we will present data concerning the influence of substrate concentration on D_{37} values obtained for X-irradiated RNA polymerase. It is also interesting to note that, at high substrate concentrations, the D_{37} value for inactivation of RNA polymerase using the synthetic polymer poly(U) is similar to the D_{37} value obtained for inactivation of the enzyme when the natural native double-stranded DNA template is used: between 34 and 36 krads.

For comparison, Figure 3 shows inactivation curves for B/r, B_{s-1}, and K12 RNA polymerases as a function of X-ray dose when assayed with the homopolymers poly(A) and poly(dA). The D_{37} value for all three enzymes when assayed with poly(A) averages 32 krads, the response being almost identical with that obtained when the assay is conducted with poly(U). Similarly, there exists a reduction in the D_{37} value for the enzyme when the assay is conducted with the analogous polydeoxyribo template; in this example, the average D_{37} value obtained from inactivation of all three enzymes using poly(dA) equaled 22 krads. However, reduction in the D_{37} value is not as extreme as was found for poly(U) and poly(dT).

BINDING EXPERIMENTS WITH HOMOPOLYMER TEMPLATES. Due to the large difference obtained in D_{37} values for the *E. coli* RNA polymerase when assayed with poly(U) and compared to results when assayed with poly(dT), at high substrate concentrations, it was possible to rationalize that the differences in radiosensitivity of the enzyme might be reflected by a

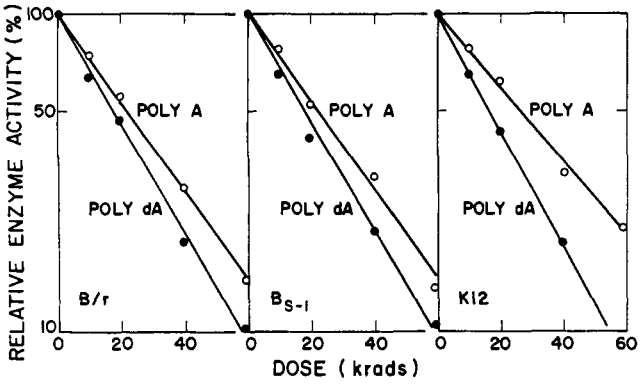

FIGURE 3: Inactivation of the enzymatic activity of *E. coli* B/r, B_{s-1}, and K12 RNA polymerases by X-irradiation when assayed with either poly(A) or poly(dA). Assays contained substrate at 375 nmol of [^{14}C]UTP (4.10×10^5 cpm/μmol) per reaction. The nonirradiated RNA polymerase samples (7.5–8.0 μg) incorporated either 4.5 nmol of [^{14}C]UMP or 6.5 nmol of [^{14}C]UMP in 20 min at 37° using either poly(A) plus Mn^{2+} or poly(dA) plus Mg^{2+}, respectively.

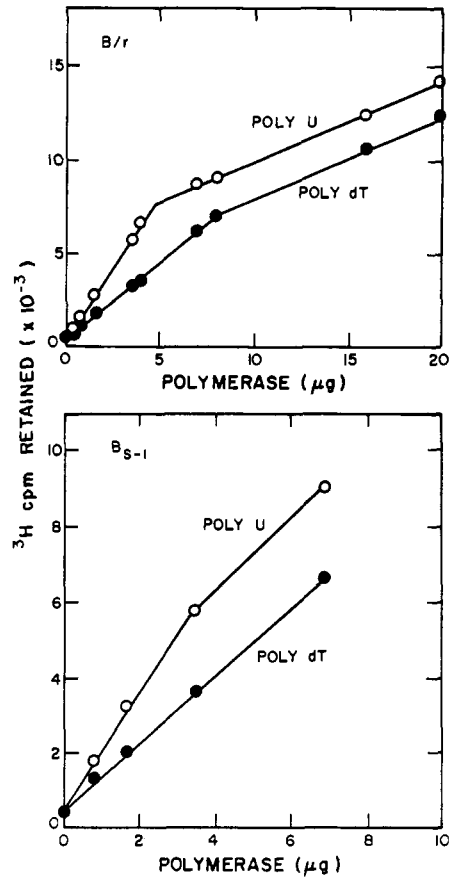

FIGURE 4: Formation of nonfilterable complexes between *E. coli* B/r (top diagram) and B_{s-1} (bottom diagram) RNA polymerases with either poly(U) or poly(dT). Reactions were prepared and assayed as described in Experimental Procedures with 2.6 nmol of either [^{3}H]poly(U) (1.1×10^4 cpm/nmol) or [^{3}H]poly(dT) (6.7×10^3 cpm/nmol) and increasing amounts of enzyme.

variable ability of the irradiated enzyme to bind to such templates. In Figure 4 control binding experiments were conducted with the B/r and B_{s-1} RNA polymerases with [^{3}H]poly(U) and [^{3}H]poly(dT) as a function of amount of enzyme present in the reaction vessel. All binding experiments described below were performed at limiting enzyme concentrations where the amount of labeled homopolymer retained on the filter was proportional to the amount of enzyme added.

In the following experiment, B/r, B_{s-1}, and K12 RNA polymerases were irradiated and assayed for their ability to bind to the homopolymers poly(U) and poly(dT). Polymer amounts used were 2.6 nmol each per reaction. Irradiated enzyme was diluted with storage buffer, and 1.5 and 3.0 μg were used with poly(U) and poly(dT), respectively. Figure 5 shows inactivation of the binding ability of the three polymerases as a function of dose for both poly(U) and poly(dT) templates. It is evident from the data presented, where the average D_{37} value is in excess of 140 krads, that reduction in binding as determined by our assay cannot account for inactivation of the RNA polymerase and, furthermore, cannot explain the difference in radiosensitivities obtained for enzymatic activity of X-irradiated RNA polymerase when assayed with various homoribo- and homodeoxyribopolymer templates.

CONTROL REACTIONS CONCERNING POLYMERIZATION RATE AS A FUNCTION OF NUCLEOSIDE TRIPHOSPHATE CONCENTRATION. It has been shown previously for a variety of homopolymer templates that the polymerization rate of RNA polymerase is a complex function of both divalent metal ion and substrate concentrations (Niyogi and Stevens, 1965a,b; Steck *et al.*, 1968; Niyogi, 1972). In general, there is not a universal divalent metal ion:substrate ratio that optimizes the polymerization rate of the RNA polymerase when assayed with various ribo- and deoxyribopolymers. For example, Steck and colleagues (1968) showed that maximum stimulation in a poly(U)-directed poly(A) synthesis reaction using *Micrococcus luteus* RNA polymerase occurred when the divalent metal ion Mn^{2+} exceeded the substrate concentration by approximately 50% and, furthermore, excess metal ion or substrate had an inhibitory effect on the polymerization process. When Mg^{2+} is present as the only divalent metal ion in the reaction mixture, very little poly(A) synthesis occurs with a poly(U) template and either *M. luteus* or *E. coli* RNA polymerases (Fox *et al.*, 1964; Niyogi and Stevens, 1965a). In control experiments concerning poly(A) synthesis utilizing a poly(U) template and *E. coli* B/r RNA polymerase, our findings agree remarkably well with the results noted above (data not shown). That is, optimum poly(A) synthesis occurs when the divalent metal ion exceeds the substrate (mole:mole) by approximately 50%, and the reaction is abortive in the presence of only Mg^{2+}. However, when either poly(dT) or poly(dU) was used as

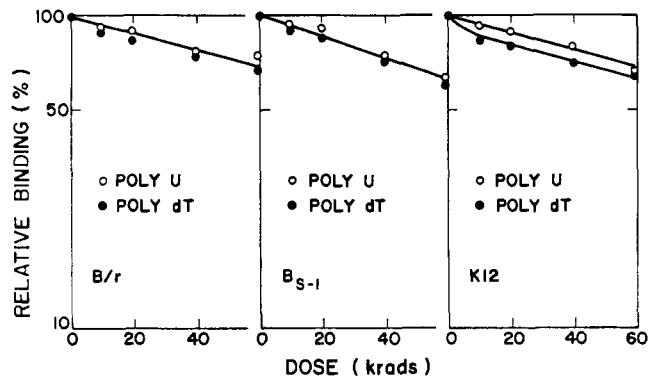

FIGURE 5: X-Ray inactivation of the binding ability of *E. coli* B/r, B_{s-1}, and K12 RNA polymerases to either poly(U) or poly(dT). The assays contained 2.6 nmol of [^{3}H]poly(U) or 2.6 nmol of [^{3}H]-poly(dT) and 1.5 μg or 3.0 μg of enzyme, respectively.

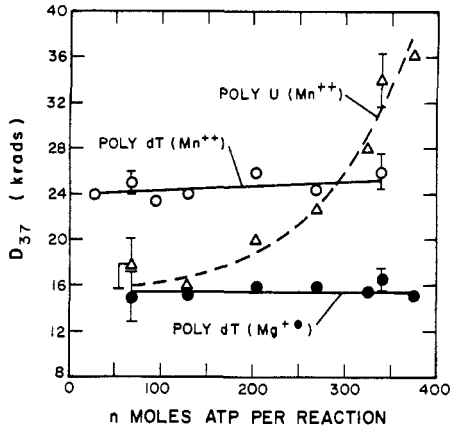

FIGURE 6: Inactivation of enzymatic activity of *E. coli* B/r RNA polymerase with X-irradiation as a function of amount of substrate (ATP) per assay reaction. The data points represent D_{37} values from the inactivation curves of the enzyme when assayed with either poly(U) plus Mn^{2+} or poly(dT) plus Mn^{2+} or Mg^{2+}. Data points with arrow bars ($\pm \sigma$ or the standard deviation) represent the average of three or more independent experiments.

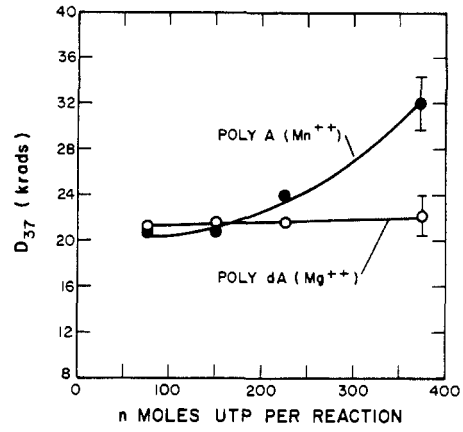

FIGURE 7: Inactivation of enzymatic activity of *E. coli* B/r RNA polymerase when assayed with either poly(A) or poly(dA) as a function of amount of substrate (UTP) per reaction. The divalent metal ion used for either poly(A)- or poly(dA)-directed reactions was Mn^{2+} or Mg^{2+}, respectively. Data points with arrow bars represent three or more independent experiments.

template in poly(A) synthesis reactions using the B/r RNA polymerase, there did not exist an optimum divalent metal ion:substrate concentration ratio (data not shown). At a fixed divalent metal ion concentration, increasing amounts of substrate (ATP) added to the reaction mixture stimulated the polymerization reaction. Our results for the poly(dT)-directed poly(A) synthesis reactions agree with the recent findings of Niyogi (1972). It is also interesting to note that either Mg^{2+} or Mn^{2+} sufficed as the divalent metal ion cofactor in poly(dT)- or poly(dU)-directed reactions.

Complexities that exist in rates of polymerization as a function of substrate concentration and divalent metal ion used have been associated with the ability of the enzyme to initiate such reactions. In the case of the poly(U) template, the metal ion quantitatively influences the specificity of chain initiation and influences the kinetics of chain polymerization (Steck *et al.*, 1968). Furthermore, it has been shown recently (Niyogi, 1972) that transcription of poly(U) templates is completely dependent on the σ factor and that transcription of poly(dT) and poly(dA) is greatly stimulated by the presence of the σ factor. The reason(s) for this requirement has been associated with the apparent polymer template configuration; that is, the melted or unstacked configurations present in single stranded homopolymers presumably lead to poor initiation in the absence of σ, especially at low substrate concentrations. Therefore, the rate of RNA synthesis is a complex function containing several variables including substrate and divalent metal ion concentrations, chemical and physical nature of the polymer template, and physical state of the enzyme.

FUNCTION OF SUBSTRATE CONCENTRATION USING A VARIETY OF HOMOPOLYMER TEMPLATES. In the following experiments, B/r RNA polymerase, X-irradiated in 0.01 M Tris buffer–0.15 M KCl (pH 7.9), was assayed with a variety of homopolymer templates (poly(U), poly(dT), poly(A), poly(dA), and poly(dU)) as a function of amount of substrate (NTP) in the reaction mixture. Figure 6 presents the D_{37} values obtained for the B/r enzyme assayed against poly(U) and poly(dT) templates with varying amounts of ATP in the reaction mixture. Results show a substrate dependence for the D_{37} value in the synthesis of poly(A) by the B/r RNA polymerase when assayed with poly(U) in the presence of Mn^{2+}. However, there exists a substrate independence for the D_{37} value in the synthesis of poly(A) when the irradiated enzyme is assayed with poly(dT) in the presence of either Mg^{2+} or Mn^{2+}.

An analogous experiment is presented in Figure 7, where it is shown that the D_{37} value for poly(U) synthesis is substrate dependent when RNA polymerase is assayed with the homoribopolymer poly(A), while a substrate independence in the D_{37} value is obtained when the enzyme is assayed with the homodeoxyribopolymer poly(dA). In comparison, the only difference in chemical composition of poly(A) compared to poly(dA) is present in the sugar structure in the backbone of the polymer (ribose *vs.* deoxyribose for poly(A) and poly(dA), respectively). However, as has been noted previously, there exist possible differences in physical nature or secondary structure of these polymers. These unique differences in chemical and physical state of the polymer evidently influence the response of irradiated RNA polymerase in the polymerization process and account for the substrate dependence or independence in the D_{37} values for inactivation previously shown. To add more data to strengthen this viewpoint, we investigated the inactivation of the B/r RNA polymerase with respect to its ability to synthesize poly(A) using the templates poly(U) and poly(dU) at both low and high substrate concentrations. It was argued that, if the sugar residue of the polymer was the sole determinant of substrate dependence or independence for the D_{37} value of inactivation, then there should exist a dissimilar response in the inactivation process of RNA polymerase as a function of substrate concentration when the poly(U) template is compared to the poly(dU) template. As is shown in Figure 8, this argument appears to be valid, for the inactivation processes are dissimilar for the two mentioned polymers. At low substrate amounts per reaction, the ability of the RNA polymerase to polymerize poly(A) using the templates poly(U) or poly(dU) gives D_{37} values of 17 and 18 krads, respectively. However, at high substrate amounts, the response of the irradiated enzyme using the poly(U) template shows a substrate dependence, while the response of the enzyme using a poly(dU) template shows substrate independence. Therefore, it is evident from the two examples mentioned above that the properties of the template molecule influence the response of the X-

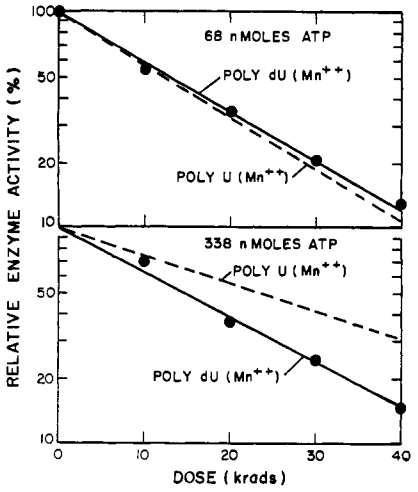

FIGURE 8: Inactivation of enzymatic activity of *E. coli* B/r RNA polymerase when assayed with a poly(dU) template. Assays were conducted at low and high substrate amounts (68 or 338 nmol of ATP per reaction) in the presence of Mn^{2+} (0.5 μmol/reaction). The dashed curves represent an analogous experiment; however, a poly(U) template was used instead of a poly(dU) template.

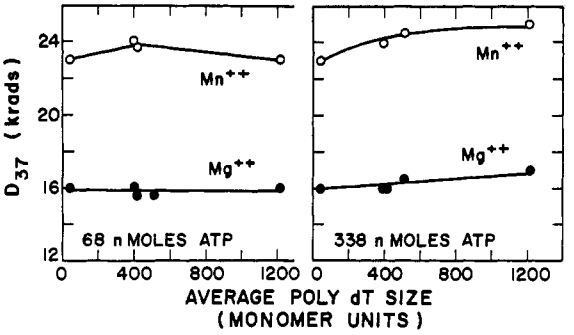

FIGURE 9: Inactivation of enzymatic activity of *E. coli* B/r RNA polymerase as a function of average monomer unit size of the poly(dT) template. Assays were performed at both low and high substrate (ATP) amounts (68 or 338 nmol/reaction) in the presence of Mn^{2+} or Mg^{2+}. Template sizes varied from an average of 39 to 1210 monomer units.

irradiated RNA polymerase with respect to its ability to catalyze *in vitro* RNA transcription.

FUNCTION OF TEMPLATE SIZE. From the preceding data it is apparent that template choice in assay of irradiated RNA polymerase influences the synthesis of RNA by the enzyme. Furthermore, since the templates we used in the experiments varied in length, it was decided to investigate the effects of poly(dT) template size on X-ray inactivation of B/r RNA polymerase. We found, in control experiments using poly(dT) templates with average lengths from 39 to 1210 monomer units, as much as a fourfold difference in the rate of poly(A) synthesis, this rate being dependent on the divalent metal ion used (G. F. Strniste, D. A. Smith, and F. N. Hayes, unpublished results). However, Figure 9 shows that there exists an independence between the D_{37} value obtained for X-irradiated RNA polymerase and template size, at low and high substrate amounts per reaction, and with two divalent metal ions. Therefore, we find no significant difference in inactivation of the B/r RNA polymerase when assayed with poly(dT) varying in average size by a factor of 30. It should be noted also that, with two different preparations of poly(U) which differed in their average monomer unit length by a factor of two (average monomer unit lengths of 227 and 421), no apparent change in the D_{37} value for inactivation of enzymatic activity of the B/r RNA polymerase was observed.

Discussion

Our data demonstrate that the radioresponse of RNA polymerase to X-irradiation is independent of the radiosensitivity exhibited by the cell from which the polymerase was isolated. Although *E. coli* strains B_{s-1}, B/r, and K12 show approximately a sixfold variance in their response to ionizing radiation, as expressed in their D_{37} values for inactivation of colony-forming ability (Pollard, 1970), the RNA polymerases isolated from these bacteria show similar, if not identical, radioresponses as expressed in their D_{37} values for inactivation of enzymatic activity. Inactivation kinetics for *in vitro* RNA polymerization are an exponential function of dose given to the enzyme for a variety of homoribo- and homodeoxyribopolymers and a natural DNA template tested. However, it was shown that the D_{37} value for inactivation of the relative enzyme activity of the RNA polymerase varied according to the template molecule used in the assay mixture at high substrate concentrations. When either poly(U) or poly(A) was used in the assay for enzymatic activity of the irradiated polymerase, the resulting D_{37} values obtained from inactivation curves were similar to the D_{37} value obtained when a natural DNA was used as a template. However, when homodeoxyribopolymers were used as templates (poly(dT), poly(dA), and poly(dU)), there resulted an increase in radiosensitivity of RNA polymerase when compared to inactivation of enzymatic activity of the polymerase utilizing a natural DNA template.

In an attempt to explain this variability in inactivation of X-irradiated RNA polymerase when assayed with various homopolymers, binding of irradiated polymerase to poly(U) and poly(dT) was examined, for it was possible to rationalize that the radioresponse of enzyme might be related to the ability of the enzyme to bind to the template molecule. However, it was found that binding of irradiated enzyme to both poly(U) and poly(dT) templates was similar and, furthermore, that the resultant D_{37} values for inactivation of binding were between four and eight times greater than D_{37} values obtained for inactivation of enzymatic activity using these two templates. Therefore, binding of the enzyme to the polymer template was excluded as the radiosensitive step in the catalytic process of RNA transcription.

When X-irradiated RNA polymerase was assayed with synthetic homopolymer templates as a function of substrate concentration, it was found that for all deoxyribopolymers tested the D_{37} values obtained were independent of amount of substrate. However, when ribopolymers were used as templates, a substrate dependence was reflected in the inactivation curves. It has been shown that, in the RNA polymerization process, the initiation step is the rate-limiting one and is most sensitive to substrate concentration fluctuations (Downey and So, 1970). Thus, in the case where there exists a substrate dependence in D_{37} values obtained from inactivation of RNA polymerase (*i.e.*, when the enzyme is assayed with homoribopolymers), it is tempting to suggest that the initiation step may be the radiosensitive target in the inactivation process. This observation will be considered with supporting evidence in the following paper.

At present it is difficult to explain the substrate indepen-

dence in the resulting D_{37} values obtained from inactivation of the RNA polymerase when assayed with homodeoxyribopolymers. However, it is interesting that the results obtained when irradiated polymerase is assayed with a poly(dT) template as a function of substrate concentration show substrate independence in the D_{37} values, although the D_{37} obtained from assay with Mn^{2+} present in the reaction mixture is 50% greater than the D_{37} obtained from the assay containing Mg^{2+}. Since there exist complexities in kinetics for the process of RNA polymerization when various template molecules are employed in the assay, it is not unlikely that irradiated RNA polymerase may respond differently according to the variables existing in the system.

Our data also demonstrate that, when the average template sizes for poly(dT) varied by a factor of 30, inactivation of enzymatic activity of the RNA polymerase by X-rays did not change significantly. This finding was also apparent for two different molecular size distributions of a poly(U) template used. Therefore, although the template composition definitely influences the inactivation process of enzymatic activity of irradiated RNA polymerase, the molecular size of such templates does not. In the following paper we demonstrate with the use of complementary oligomer primers how the initiation step in the catalytic process of RNA polymerization can account, at least in part, for the radiosensitivity of the RNA polymerase. Furthermore, we show how X-irradiated RNA polymerase can influence the fidelity of transcription.

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