

---

**Laser crosslinking of *E. coli* RNA polymerase and T7 DNA**

---

Catherine A. Harrison\*, Douglas H. Turner\* and David C. Hinkle<sup>†</sup>

---

<sup>\*</sup>Department of Chemistry, and <sup>†</sup>Department of Biology, University of Rochester, Rochester, NY 14627, USA

---

Received 1 February 1982; Accepted 3 March 1982

---

**ABSTRACT**

The first photochemical crosslinking of a protein to a nucleic acid using laser excitation is reported. A single, 120 mJ, 20 ns pulse at 248 nm crosslinks about 10% of bound *E. coli* RNA polymerase to T7 DNA under the conditions studied. The crosslinking yield depends on mercaptoethanol concentration, and is a linear function of laser intensity. The protein subunits crosslinked to DNA are  $\beta$ ,  $\beta'$  and  $\sigma$ .

**INTRODUCTION**

Photochemical crosslinking is a powerful method for studying protein-nucleic acid complexes(1,2). Pioneering work by Markovitz (3) and by Struiste and Smith(1,4) showed that covalent crosslinking of protein and nucleic acid can be induced by UV photons without additional reagents. A few elegant studies have used this technique to determine the regions of particular proteins that bind to nucleic acids(5-9). In these studies, samples were irradiated at 254 nm with a low pressure Hg lamp for times ranging from 5 to 240 min. The recent development of high power excimer lasers makes it possible to provide an equivalent number of photons in 20 ns or less. This paper reports the first crosslinking of a protein-nucleic acid complex using excimer laser radiation.

Laser crosslinking of the complex formed by *E. coli* RNA polymerase and T7 DNA has been studied. This provides an excellent model system because of the detailed studies of Wu and coworkers on the conventional UV crosslinking of this complex (8,9). We find similar crosslinking using a KrF laser operating at 248 nm. Depending on conditions, approximately 5 to 20% of DNA bound RNA polymerase is crosslinked with a single, 20 ns

pulse of 120 mJ. Higher yields should be possible at higher radiation fluxes. The results demonstrate that laser crosslinking can rapidly induce covalent bonds between proteins and nucleic acids, and suggest several applications in which the technique will provide unique information.

### MATERIALS AND METHODS

Preparation of RNA polymerase and T7 DNA. RNA polymerase was purified from *E. coli* B by the procedure of Burgess and Jendrisak(10). Electrophoresis on an acrylamide gel in the presence of sodium dodecyl sulfate (SDS) indicated the enzyme was greater than 90% pure and contained 0.8 equivalents of sigma subunit.

[<sup>3</sup>H]-T7 DNA was prepared as described previously(11).

The concentration of RNA polymerase was determined by the method of Lowry et al(12) using bovine serum albumin as standard and correcting by a factor of 0.8 to give true weight(13). Molar concentrations were calculated assuming a molecular weight of  $4.5 \times 10^5$  for the holoenzyme and  $2.6 \times 10^7$  for T7 DNA.

Photocrosslinking. Unless otherwise indicated, T7 [<sup>3</sup>H]-DNA and *E. coli* RNA polymerase were irradiated in 1.5 ml, conical, polypropylene test tubes (Eppendorf) in 0.1 ml of the following binding buffer: 10 mM Tris, pH 8.0, 10 mM MgCl<sub>2</sub>, 50 mM NaCl, 10 mM mercaptoethanol (HSEtOH), 1 mM ethylene diamine tetraacetic acetate (EDTA). The diameter of the tube at the surface of the sample was 5 mm and maximum depth was 8 mm. Samples were irradiated with either a low pressure Hg lamp or a KrF laser. The laser was focused to a 5 x 2 mm spot with a 15 cm focal length lens. The light intensity of the lamps was measured with an International Light model 254 germicidal photometer with a PT-100 detector head containing a narrow bandpass filter for 254 nm. The laser intensity was measured with a Gen Tec PRJ-D joulemeter with ED-200 pyroelectric detector head. A ferric oxalate actinometer was used to compare the two detectors(14,15). The laser intensity measured by actinometry was approximately 95% that measured by the joulemeter. However, the lamp intensity measured by actinometry was about 50% greater than measured by the photometer. This is probably due to photons at wavelengths

other than 254 nm. These corrections have not been applied to the reported intensities. Thus the energy of the lamp is underestimated to the extent that reaction is induced by wavelengths other than 254 nm.

Energy doses depended on time of exposure for the lamps and on energy per pulse for the laser. Typical, uncorrected doses were  $185 \text{ ergs/mm}^2\text{-s}$  for the Hg lamps and  $120 \text{ mJ/pulse}$  for the laser. Some samples were irradiated after being "purged" with  $\text{N}_2$ . In these cases, a hypodermic needle in the side of the Eppendorf tube carried water-saturated  $\text{N}_2$  over the sample for 60 minutes prior to and also during irradiation.

Filter Binding Assay. The nitrocellulose filter assay of Hinkle and Chamberlin(11) was used to determine the amount of DNA bound to RNA polymerase. For measurements of noncovalent binding, RNA polymerase was incubated for 10 min at  $37^\circ\text{C}$  with  $1.7 \text{ }\mu\text{g}$  of T7 [ $^3\text{H}$ ]-DNA ( $2.5 \times 10^4 \text{ cpm}/\mu\text{g}$ ) in  $0.1 \text{ ml}$  binding buffer ( $10 \text{ mM Tris}$ ,  $\text{pH } 8.0$ ,  $10 \text{ mM MgCl}_2$ ,  $50 \text{ mM NaCl}$ ,  $10 \text{ mM HSEtOH}$ ,  $1 \text{ mM EDTA}$ ). Samples were then diluted with  $3 \text{ ml}$  binding buffer, immediately filtered through  $24 \text{ mm}$  nitrocellulose filters, and the radioactivity on the dried filters determined in a liquid scintillation counter. For measurements of light induced cross-linking,  $0.1 \text{ ml}$  samples were diluted to  $1 \text{ ml}$  with binding buffer containing  $1\text{M NaCl}$ , filtered, and the filters were washed with  $3 \text{ ml}$  of binding buffer with  $1\text{M NaCl}$  followed by  $3 \text{ ml}$  of binding buffer. Filters were then dried and radioactivity was determined in a liquid scintillation counter. The binding curves were fit to the equation(11):

$$R = 1 - e^{-bx} \quad (1)$$

where R is the fraction DNA retained, x is the molar ratio of RNA polymerase to T7 DNA, and b is a constant representing the fraction of RNA polymerase molecules bound to DNA in solution and the efficiency with which these complexes are trapped by the nitrocellulose filter.

Analysis of Photoproducts. To determine which protein subunits were bound to DNA, the photoproducts were treated as described by Hillel and Wu(8) to digest free DNA. Samples were first incubated for  $30 \text{ min}$  at  $37^\circ$  with  $2 \text{ }\mu\text{g}$  pancreatic DNase I

(Sigma) per  $\mu\text{g}$  DNA. This was followed with a 30 min incubation at  $37^\circ$  with  $0.6 \mu\text{g}$  phosphodiesterase (Boehringer) per  $\mu\text{g}$  of DNA. The last incubation was for 60 min at  $37^\circ$  with  $0.2 \mu\text{g}$  alkaline phosphatase (Boehringer) per  $\mu\text{g}$  DNA and  $0.6$  unit *Staphylococcus aureus* nuclease (Boehringer) per  $\mu\text{g}$  of DNA in the presence of  $0.01\text{M}$   $\text{CaCl}_2$ . After the DNA was digested, samples were chilled to  $0^\circ$  and precipitated with cold 10% trichloroacetic acid. The samples were then centrifuged at  $15,000 \text{ g}$  for 15 min. The pellets were washed once with cold 10% trichloroacetic acid and twice with cold acetone. Acetone was removed by blowing  $\text{N}_2$  over the pellets. The samples were denatured in electrophoresis buffer containing 1% SDS by heating on a boiling water bath for 90 s.

Electrophoresis of the proteins was done in 5 mm diameter, 14 cm long, 7.5% polyacrylamide tube gels using a Laemmli discontinuous buffer system (16). Gels were subsequently stained with Coomassie Blue (17). To determine the radioactivity profile, gels were cut into 1 mm slices. Three slices were placed in each glass scintillation vial containing  $0.1 \text{ ml H}_2\text{O}$ , 1 ml NCS tissue solubilizer (Amersham), and 10 ml liquid scintillation fluid (PPO-POPOP, Amersham). The vials were sealed, incubated at  $50^\circ$  for 6 hours and then counted in a Beckmann Scintillation counter.

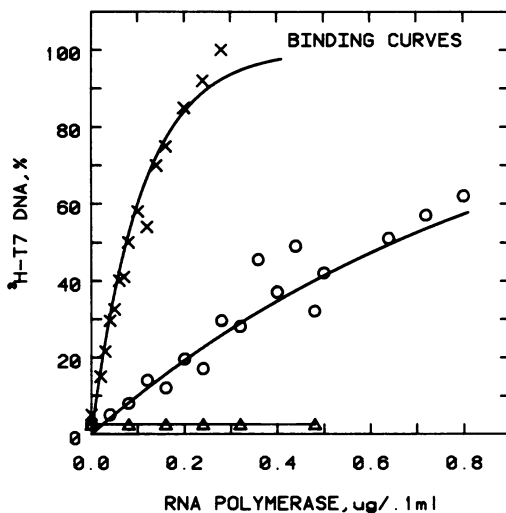
Separation of  $\beta$  and  $\beta'$  subunits. To separate  $\beta$  and  $\beta'$  subunits, 8% polyacrylamide gels were made, except that the acrylamide/bis ratio was 300/1. Gels were run at  $0.5 \text{ mamp/tube}$  for 28 hours, and the marker dye and  $\alpha$  subunit were allowed to run off the gel. This procedure resulted in a 2 mm separation between the  $\beta$  and  $\beta'$  subunits. Gels were cut into 1 mm slices, 2 slices were placed in each vial, and samples were then treated as previously described.

## RESULTS

The binding of *E. coli* RNA polymerase to T7 DNA has been measured using the nitrocellulose filter assay (11). In solutions of low ionic strength, the RNA polymerase binds very tightly to a number of high affinity sites on the T7 DNA molecule. DNA molecules that are bound to at least one enzyme molecule are efficiently trapped by nitrocellulose filters

(Fig. 1). However, if these noncovalent RNA polymerase-T7 DNA complexes are diluted in 1 M NaCl prior to filtration, the complexes dissociate and only a background level of about 2% of the DNA is retained on the filter. Thus the fraction DNA retained at 1M NaCl provides a simple assay for covalent complexes produced by photochemical crosslinking.

To determine the efficiency of crosslinking induced by a single, 120 mJ, 20 ns, 248 nm laser pulse, 0.1 ml samples containing 1.7  $\mu\text{g}$  DNA and varying amounts of RNA polymerase were irradiated and then assayed at 1M NaCl. Samples irradiated in 1M NaCl gave background levels of retention, as did samples in which the DNA and protein were irradiated separately at 50 mM NaCl and then mixed. However, significant percentages of DNA were retained when samples containing DNA and polymerase were



**Figure 1.** Nitrocellulose filter assay for the binding of RNA polymerase to T7 DNA. Samples contained 1.7  $\mu\text{g}$  of T7 [ $^3\text{H}$ ]-DNA and the indicated amounts of enzyme in 0.1 ml. The solid lines through o's and x's are least squares fits to:  $R=1-e^{-bx}$  where  $x$  is the molar ratio of RNA polymerase to DNA. (X) Samples filtered in binding buffer to assay noncovalent binding. Fitted  $b = 0.3$ . (O) Samples in binding buffer irradiated with a 120 mJ laser pulse, and filtered in 1 M NaCl as described under Methods. Fitted  $b = 0.03$ . ( $\Delta$ ) Samples in binding buffer containing 1 M NaCl irradiated with a 120 mJ laser pulse and filtered in 1 M NaCl.

irradiated at 50 mM NaCl where protein-DNA complexes form (Fig. 1). If noncovalent complexes in 50 mM NaCl and covalent complexes in 1M NaCl are retained at equal efficiency,\* then the initial slopes of the binding curves in Fig. 1 can be used to calculate the fraction of DNA bound enzyme that is crosslinked by a single, 120 mJ laser pulse. The ratio of these slopes is 0.1. Thus about 10% of the protein is crosslinked.

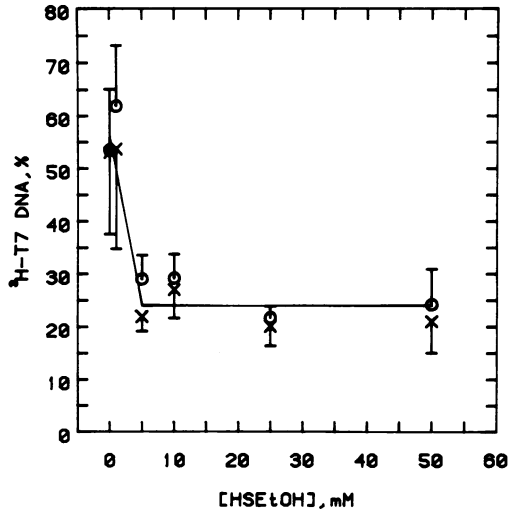
One potential photochemical mechanism for the crosslinking is the generation of a free radical (19,20). If this radical had a long lifetime, many of the advantages of using a short laser pulse would be negated. Long lived free radicals can be quenched using scavengers such as HSEtOH and the experiments described above employed 10 mM HSEtOH. To gain some insight into the photochemistry, crosslinking was also measured as a function of HSEtOH concentration. Low salt (50 mM NaCl) filter binding curves were measured at 0, 1, 10, and 50 mM HSEtOH, and were identical within experimental error. Samples containing 1.7  $\mu$ g DNA and 0.48  $\mu$ g RNA polymerase were then irradiated and assayed at 1M NaCl. The results are shown in Fig. 2 as a function of HSEtOH concentration. The fraction of DNA retained drops roughly a factor of 2 as HSEtOH concentration is increased from 0 to 5 mM, and then levels off. Evidently, at least two processes are responsible for crosslinking, and one of them is not quenched by HSEtOH.

Fig. 2 also shows the effect of purging the solutions with  $N_2$ . The purged samples consistently have somewhat less crosslinking (see also Fig. 4), but the effect is within experimental error.

In principle, the data in Figs. 1 and 2 can be used to esti-

---

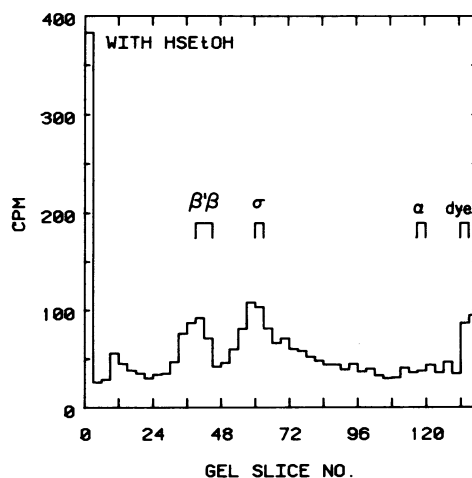
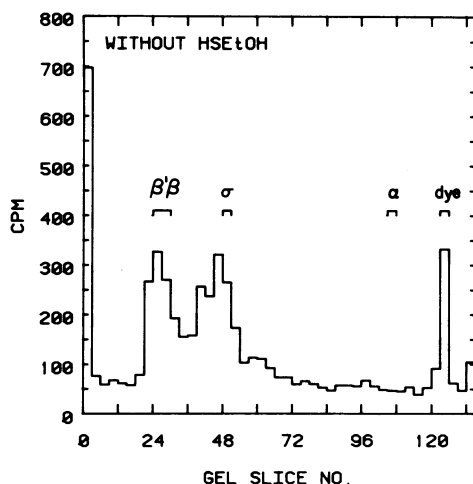
\*Footnote: RNA polymerase is quantitatively retained by nitrocellulose filters during filtration in both 50 mM and 1M NaCl (11,18). Thus it seems likely that DNA which is covalently attached to an RNA polymerase molecule will be retained with an efficiency of 100%. In the experiment presented in Fig. 1, we estimate an apparent efficiency for the trapping of noncovalent RNA polymerase-DNA complexes of about 30%. However, with other preparations of *E. coli* RNA polymerase, this efficiency is close to 100% (11). It seems likely that about 70% of the RNA polymerase molecules in our current preparation are inactive and unable to form stable complexes with T7 DNA.



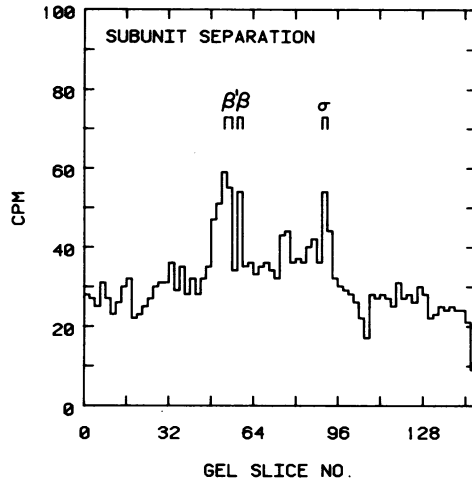
**Figure 2.** Crosslinking of RNA polymerase to T7 DNA as a function of HSEtOH concentration. Samples (0.1 ml) containing 1.7  $\mu\text{g}$  T7 [ $^3\text{H}$ ]-DNA, 0.48  $\mu\text{g}$  E. coli RNA polymerase, and the indicated concentrations of HSEtOH were irradiated with a 120 mJ laser pulse, and filtered in 1 M NaCl as described under Methods. Values are means  $\pm 1$  standard deviation of 4 to 10 determinations. (X) Samples purged by flowing water saturated nitrogen over them for 60 min. Nitrogen continued to flow over the samples during the irradiation. (O) Samples not purged.

mate the  $k_q \tau$  product for the reactive state quenched by HSEtOH. The relevant equation is  $f_0/f_q = 1 + k_q \tau [\text{HSEtOH}]$  (21-23). Here  $f_0$  and  $f_q$  are the fractions of reaction without and with added quencher, respectively;  $k_q$  is the rate of quenching and  $\tau$  is the lifetime of the reactive state. When the data are plotted this way, there is a lot of scatter. This is not surprising considering the indirect nature of the measurement. Nevertheless, the slope at low concentrations of HSEtOH appears to be between 100 and 400  $\text{M}^{-1}$ . This provides an approximate value for  $k_q \tau$ . The lifetime,  $\tau$ , could be estimated if  $k_q$  was known. Typical values of  $k_q$  for fluorescence quenching of proteins by acrylamide range from  $1 \times 10^8$  to  $4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  (24,25). Assuming similar rates for quenching of the RNA polymerase-DNA complex, this suggests the lifetime of the quenchable, reactive species is in the range 25 to 4000 ns.

The above results indicate that about 1/2 of the crosslinking observed at HSEtOH concentrations of 1 mM and less is due to a reactive species that may have a significant lifetime. In an elegant study, Hillel and Wu recently used crosslinking with a germicidal lamp to show that in nonspecific complexes, T7 DNA is in contact with the  $\beta$ ,  $\beta'$  and  $\sigma$  subunits of E. coli RNA polymerase(8). Their work was done in the absence of a reducing agent. The bipartite nature of the photochemistry suggested it was worthwhile to repeat some of their experiments in the presence of HSEtOH using laser excitation. Laser experiments were







**Figure 3.** SDS Polyacrylamide gel analysis of crosslinked T7 [ $^3\text{H}$ ]-DNA and RNA polymerase.

**3a** Samples contained 1.6  $\mu\text{g}$  T7 [ $^3\text{H}$ ]-DNA and 6.7  $\mu\text{g}$  RNA polymerase in 0.1 ml binding buffer with 20 mM Tris, pH 8.0, and no HSEtOH. Six separate samples were each irradiated with one 120 mJ laser pulse (248 nm) and were subsequently combined into one sample. The sample was treated with DNase and analyzed on a 7.5% SDS-polyacrylamide gel as described under Methods. The protein subunits as they appeared in the stained gel are indicated on the graph.

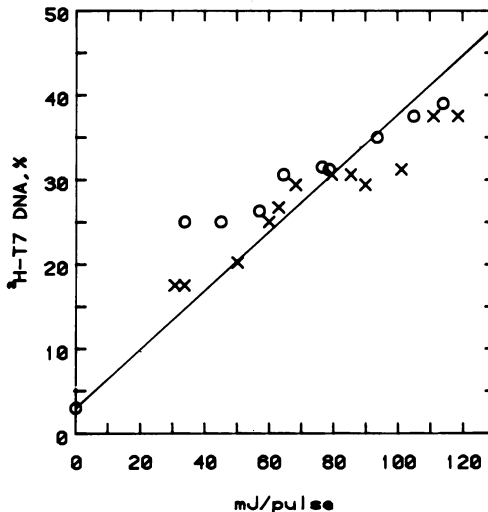
**3b** Samples contained 1.7  $\mu\text{g}$  T7 [ $^3\text{H}$ ]-DNA and 26.7  $\mu\text{g}$  RNA polymerase in 0.1 ml binding buffer. Three separate samples were each irradiated with one 100 mJ laser pulse (248 nm) and were combined into one sample, which was treated as in Fig. 3a.

**3c** Samples contained 1.7  $\mu\text{g}$  T7 [ $^3\text{H}$ ]-DNA and 26.7  $\mu\text{g}$  RNA polymerase in 0.1 ml binding buffer. Three separate samples were each irradiated with one 100 mJ laser pulse (248 nm) and were combined into one sample. The sample was electrophoresed on an 8% polyacrylamide gel with an acrylamide/bis ratio of 300/1 to separate the  $\beta$  and  $\beta'$  subunits. Due to the long duration of the electrophoresis, both the  $\alpha$  subunit and the dye marker ran off the gel. The other subunits are shown in the graph.

also done in the absence of HSEtOH to see if the crosslinking pattern corresponded to that obtained by Hillel and Wu using a lamp. The procedure for identifying the crosslinked subunits is described under Methods. The radioactivity profiles are shown in Fig. 3. The results demonstrate crosslinking of T7

DNA to the  $\beta$ ,  $\beta'$ , and  $\sigma$  subunits, but not to  $\alpha$ . This is in agreement with the results of Hillel and Wu (8), and indicates the finite lifetime of the quenchable species is not a problem at this level of resolution.

For the experiments described above, the laser power density at the sample was  $6 \times 10^7$  W/cm<sup>2</sup>. At this high intensity, there is the possibility that multiphoton events will be important (26,27). If they are, then the fraction of RNA polymerase crosslinked may not be linearly dependent on the laser intensity. To determine the intensity dependence of crosslinking, samples containing 0.48  $\mu$ g RNA polymerase and 1.7  $\mu$ g T7 [<sup>3</sup>H]-DNA in 0.1 ml were irradiated with single laser pulses of varying energy. Figure 4 shows the results of high salt filter binding assays on these samples. Within experimental error, the percentage DNA retained is linearly dependent on laser intensity. Since the percentage DNA retained (15 to 40%) falls within a region of the low salt binding curve that is essentially linear (see Figure 1),



**Figure 4.** Dependence of crosslinking on energy/laser pulse. Samples contained 1.7  $\mu$ g [<sup>3</sup>H]-T7 and 0.48  $\mu$ g RNA polymerase in 0.1 ml binding buffer. Samples were irradiated with single laser pulses at 248 nm and were filtered in 1 M NaCl as described under Methods. (X) Samples purged by flowing water saturated nitrogen over them for 60 min. Nitrogen continued to flow over the samples during the irradiation. (O) Samples not purged.

this indicates the fraction RNA polymerase crosslinked is also linear with laser intensity. Thus there is no evidence for multiphoton processes.

A comparison of DNA retained on filters, after irradiation by the KrF laser or a germicidal lamp also gives no indication of multiphoton events. Samples of 0.1 ml containing 1.7  $\mu\text{g}$  T7 DNA, 1.1  $\mu\text{g}$  RNA polymerase, 10 mM HSEtOH, 50 mM NaCl were irradiated with a single 120 mJ laser pulse, or with 150 mJ from a germicidal lamp. The amounts of DNA retained in the 1M NaCl filter binding assay were 59 and 67% for the laser and lamp, respectively. Evidently, the quantum efficiencies for laser and lamp are comparable. This suggests that both induce the same, single photon chemistry under these conditions. The results also indicate that crosslinking induced by the laser pulse is not limited by saturation effects, even at the highest power densities used.

The large power density of the laser pulse is a potential disadvantage, if it results in a larger quantum yield for damage to the macromolecules. The problem is not artifacts due to binding of damaged molecules. As long as a single pulse is used, damaged molecules will not have time to bind and absorb a second photon for crosslinking. However, damaged molecules may affect subsequent analysis of the crosslinked complex. For example, strand scission might make it difficult or impossible to do nucleic acid sequencing after crosslinking. To preliminarily assess damage to the DNA, equilibrium sedimentation was used to determine the average number of strand breaks induced per T7 molecule. Samples of 1.7  $\mu\text{g}$  DNA in 0.1 ml were irradiated either with 120 mJ from a single laser pulse, or 150 mJ from the germicidal lamp. They were then run on 5 to 20% alkaline sucrose gradients. The average number of strand breaks,  $p$ , was calculated from the equation of Van der Schans et al (28).

$$\left(\frac{S_p}{S_m}\right)^{1/a} = \frac{p+5-a}{2p} - \frac{1}{2p} [p^2 + (2a+2)p + (9-2a+a^2)]^{1/2}$$

Here  $S_p$  and  $S_m$  are the sedimentation coefficients for broken and unbroken molecules, respectively, and  $a$  is taken as 0.53 (29). Typical sedimentation patterns from one experiment are shown in

Fig. 5. Experiments were done in 10 mM Tris, pH 8, and 20 mM citrate, pH 6.2, buffers. In different experiments, the average number of strand breaks ranged from 1 to 6, and 3 to 24 for lamp and laser irradiation, respectively. For a given experiment with similar doses, the laser radiation always induced more breaks than the lamp. Evidently, the quantum yield for strand scission is dependent on light intensity. A similar effect has been noticed previously using UV lamp excitation (30). Addition of glucose or purging with nitrogen did not inhibit the laser induced breakage.

To assay for possible protein damage, 2.1  $\mu\text{g}$  of RNA polymerase was irradiated with a 120 mJ pulse, and run on a 7.5% polyacrylamide gel in 0.1% SDS (16). The  $\alpha$ ,  $\beta/\beta'$ , and  $\sigma$  subunits all had the same mobility as for an unirradiated control and no new bands were observed. Thus, at this level of resolution,

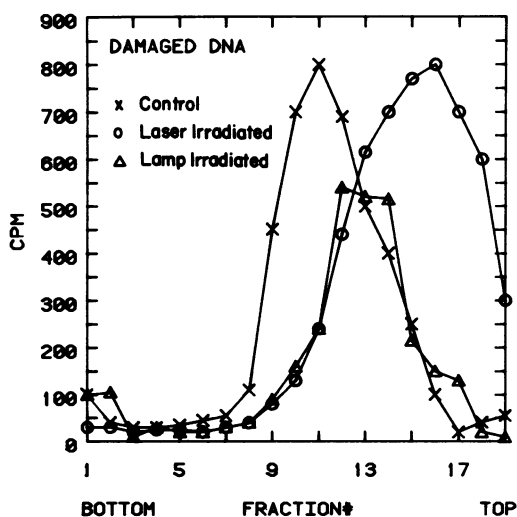


Figure 5. Sedimentation profiles of irradiated and unirradiated DNA. Samples containing 1.7  $\mu\text{g}$  of T7 [ $^3\text{H}$ ]-DNA in 0.1 ml of binding buffer were irradiated with 120 mJ from a single laser pulse or 150 mJ from a germicidal lamp. 0.3  $\mu\text{g}$  of DNA from each sample was layered on a 5-10% alkaline sucrose gradient, and centrifuged at 50,000 rpm for 90 min at 5°C in an SW 60 rotor. The gradient was fractionated into 0.2 ml portions to which 2 ml of scintillation fluid were added. Radioactivity was determined in a liquid scintillation counter. ( $\Delta$ ) Lamp irradiated, (O) Laser irradiated, (X) No irradiation.

no protein damage is observed.

#### DISCUSSION

This paper presents the first analysis of laser crosslinking of a protein-nucleic acid complex. The results demonstrate that high yields can be obtained. With *E. coli* RNA polymerase, about 10% of the bound protein can be crosslinked to T7 DNA with a single, 20 ns, 120 mJ pulse. The fraction of protein reacted increased linearly with intensity, indicating higher yields should be obtainable with more powerful lasers. KrF lasers with outputs of 1.5 J/pulse are available commercially, and systems emitting more than 50J/pulse have been reported (31). Presumably the yield will eventually be limited by saturation of the absorption responsible for the photochemistry, or by the onset of dielectric breakdown in the solution. However, it is not unreasonable to expect that 25% of bound protein can be crosslinked with a single pulse. This suggests the possibility of simultaneously crosslinking to DNA several proteins in a multienzyme complex. For example, this could provide unique information on the interrelationships of proteins involved in replication (32).

The experiments reported provide several insights into the photochemistry of the laser crosslinking. The linear dependence of crosslinking with intensity suggests a single photon process is involved. The similar yields obtained using the laser and Hg lamp, and the observation that the same subunits are crosslinked with both sources are also consistent with single photon excitation. The strong dependence on the concentration of the free radical scavenger, HSEtOH, indicates at least two classes of reactive species are formed. The first class presumably contains free radicals that are quenched by HSEtOH. The second class is not quenched by HSEtOH, suggesting it is not a long lived free radical, or that it is completely protected from diffusive quenching.

One of the advantages of laser crosslinking is the short irradiation time. In principle, it can be used to "freeze" intermediates in any 20 ns interval. The results with HSEtOH indicate that addition of a scavenger is necessary to assure that diffusion of long lived radicals does not give artifactual

---

linking. Previous work on the crosslinking of E. Coli RNA polymerase and T7 DNA in nonspecific complexes showed that  $\beta$ ,  $\beta'$ , and  $\sigma$  subunits were crosslinked to the DNA in the absence of radical scavenger (8). Using laser excitation, this work was repeated in the presence of HSEtOH to determine if the long lived species affected the results. Crosslinking patterns identical with those of Hillel and Wu (8) were observed. Thus, diffusion of the long lived species is not important at this level of resolution. This indicates the lifetime of this species is too short for major conformational fluctuations of the protein or nucleic acid. However, it is possible that problems due to diffusion of reactive species may arise at a more microscopic level of analysis.

One disadvantage of the laser method is that it induces somewhat more chain scission in DNA than does Hg lamp irradiation. This will complicate attempts to combine laser crosslinking with nucleic acid sequencing methods.

Due to the  $10^{10}$  or more increase in photon flux, lasers have several advantages over Hg lamps for the study of protein-nucleic acid interactions. Considerably less time is required to generate quantities of crosslinked complexes sufficient for study. Thus it should be possible to "freeze" intermediates generated in rapid reactions such as restriction enzyme cutting (33,34). Moreover, artifacts due to crosslinking of UV damaged molecules should be suppressed by the use of single pulses. Very few protein and nucleic acid conformational changes occur on a time scale of 20 ns so that absorption of a second photon following a light induced conformational change is unlikely (35,36). The short irradiation time will also be important for in vivo studies, since long exposures to UV irradiation affect the entire metabolism of a cell. Finally, it may be possible to simultaneously crosslink several proteins that are operating in concert. The results reported here indicate there are no fundamental limitations to the realization of this potential.

Acknowledgements We thank J. Wilson, D. Ham, D. Brown, G. Flynn, J. Ahl, and S. Formicola for help with preliminary laser experiments; M. DeWyngaert for the initial RNA polymerase prep-

aration; L. Prakash for the use of her photometer and UV irradiation apparatus; and J. Zengel for the gel conditions used to separate  $\beta$  and  $\beta'$ . This work was supported by National Institutes of Health grants GM 28533 and GM 21782. DHT is an Alfred P. Sloan Fellow and DCH is recipient of Public Health Service Career Program Award GM 00221.

#### REFERENCES

1. Smith, K. C. (1976) in Photochemistry and Photobiology of Nucleic Acids, Wang, W. Y., Ed., Vol. II, pp. 187-218, Academic Press, New York.
2. Schimmel, P. R. and Budzik, G. P. (1977) Methods in Enzymology, Jakoby, W. B. and Wilchek, M., Eds., Vol. 46, pp. 168-180, Academic Press, New York.
3. Markovitz, S. (1972) Biochim. Biophys. Acta 281, 522-534.
4. Struiste, G. F. and Smith, D. A. (1974) Biochemistry 13, 485-493.
5. Yue, V. T. and Schimmel, P. R. (1977) Biochemistry 16, 4678-4684.
6. Lica, L. and Ray, D. S. (1977) J. Mol. Biol. 115, 45-59.
7. Paradiso, P., Nakashima, Y. and Konigsberg, W. (1978) in Biomolecular Structure and Function, Agris, P. F., Ed., pp. 581-587, Academic Press, New York.
8. Hillel, Z. and Wu, C. W. (1978) Biochemistry 17, 2954-2961.
9. Park, C. S., Hillel, Z. and Wu, C. W. (1980) Nucleic Acids Res. 8, 5895-5912.
10. Burgess, R. R. and Jendrisak, J. J. (1975) Biochemistry 14, 4634-4638.
11. Hinkle, D. C. and Chamberlin, M. J. (1972) J. Mol. Biol. 70, 157-185.
12. Lowry, O., Rosebrough, N., Farr, A., and Randall, R. (1951) J. Biol. Chem. 193, 265-275.
13. Berg, D., Barrett, K., and Chamberlin, M. (1971) Methods in Enzymology 21, 506-519.
14. Hatchard, C. G. and Parker, C. A. (1956) Proc. Roy. Soc. (London) A235, 518-536.
15. Murov, S. L. (1973) Handbook of Photochemistry, 119-123, Marcel Dekker, N.Y.
16. Laemli, U. K. (1970) Nature (Lond.) 227, 680-685.
17. Fairbanks, G., Steck, T. C., and Wallach, D. F. H. (1971) Biochemistry 10, 2606-2617.
18. Strauss, H. S., Boston, R. S., Record, M. T., and Burgess, R. R. (1981) Gene 13, 75-87.
19. Jellinek, T., and Johns, R. B. (1970) Photochem. Photobiol. 11, 349-359.
20. Smith, K. C. (1970) Biochem. Biophys. Res. Commun. 39, 1011-1016.
21. Stern, O., and Volmer, M., (1919) Physik, Z., 20, 183-188.
22. Parker, C. A. (1968) Photoluminescence of Solutions, 72-74, Elsevier, N.Y.
23. Turro, N. J. (1978) Modern Molecular Photochemistry, 246-248, Benjamin, Menlo Park, Cal.

24. Eftink, M. R. and Ghiron, C. A. (1976) *Biochemistry* 15, 672-680.
25. Eftink, M. R. and Ghiron, C. A. (1977) *Biochemistry* 16, 5546-5551.
26. McClain, W. M. (1974) *Acc. Chem. Res.* 7, 129-135.
27. Eberly, J. H. and Lambropoulos, P., ed. (1978) *Multiphoton Processes*, J. Wiley, N.Y.
28. Van der Schans, G. P., and Aten, J. B. T., and Blok, J. (1969) *Analytical Biochemistry* 32, 14-30.
29. Burgi, E., and Hershey, A. D. (1963) *Biophys. J.* 3, 309-321.
30. Rahn, R. P. and Patrick, M. H. (1976) in *Photochemistry and Photobiology of Nucleic Acids*, Wang, S. Y., Ed., Vol.II, 97-145, Academic Press, New York.
31. Rhodes, C. K., ed. (1979) *Excimer Lasers*, Springer-Verlag, N.Y.
32. Alberts, B., Barry, J., Bittner, M., Davies, M., Hama-Inaba, H., Liu, C., Mace, D., Moran, L., Morris, C. F., Piperno, J., and Simha, N. K. (1977) in *Nucleic Acid-Protein Recognition*, Vogel, H. J., Ed., 31-63, Academic Press, N.Y.
33. Modrich, P. and Rubin, R. A. (1977) *J. Biol. Chem.* 252, 7273-7278.
34. Modrich, P. and Zabel, D. (1976) *J. Biol. Chem.* 251, 5866-5874.
35. Careri, G., Fasella, P., and Gratton, E. (1975) *CRC Crit. Rev. Biochem.*, 141-164.
36. Pörschke, D. (1977) in *Chemical Relaxation in Molecular Biology*, Pecht, I. and Rigler, R., Eds., 191-218, Springer-Verlag, N.Y.