Proc. Nat. Acad. Sci. USA
Vol. 69, No. 10, pp. 2855–2859, October 1972

Uncoupling of the *recBC* ATPase from DNase by DNA Crosslinked with Psoralen

(DNA-dependent ATPase/DNA intercalating agents/exonuclease V/*E. coli*)

ALEXANDER E. KARU AND STUART LINN

Department of Biochemistry, University of California, Berkeley, Calif. 94720

Communicated by W. Z. Hassid, July 31, 1972

ABSTRACT **Exonucleolytic cleavage of DNA by the *recBC* DNase is accompanied by a DNA-dependent ATP hydrolysis that ceases when the DNA has been digested to a limit. On the other hand, DNA that has been crosslinked by 4,5′,8-trimethylpsoralen in the presence of 360-nm light remains an effective cofactor in the ATPase reaction, but is resistant to digestion by the enzyme. Psoralen-treated DNA is degraded by pancreatic DNase, micrococcal nuclease, and *Escherichia coli* B restriction enzyme, but not by *Neurospora crassa* nuclease, suggesting that crosslinking did not grossly distort the duplex structure of the DNA. The psoralen-DNA is not a potent inhibitor, but competes with single-stranded DNA from bacteriophage fd for the *recBC* DNase to roughly the same extent as does normal duplex DNA. DNA treated with psoralen in the dark, exposed to 360-nm light in the absence of psoralen, or treated with the intercalating agents ethidium bromide, 9-aminoacridine, ICR-191, or actinomycin D, responds to the enzyme no differently from untreated DNA. However, DNA crosslinked with mitomycin C or nitrogen mustard behaves similarly to psoralen-treated DNA. The relationship of these findings to models for the function and control of the *recBC* ATPase and nuclease, and the advantages of psoralen as a DNA crosslinking agent, are discussed.**

The *recB* and *recC* genes of *Escherichia coli* control a deoxyribonuclease assumed to function in DNA recombination and rapair. This enzyme degrades duplex DNA exonucleolytically to short oligonucleotides and also catalyzes a DNA-dependent hydrolysis of ATP to ADP and Pi (1, 2). Furthermore, ATP-dependent DNases from other microorganisms have been reported that possess DNA-dependent ATPase activity (3–6). The ATPase reaction of these enzymes is curious in that up to 40 ATP molecules can be hydrolyzed per DNA phosphodiester bond broken, with the exact ratio depending upon the reaction conditions (3–7). In addition, there is no theoretical energy requirement for catalysis of the exothermic DNase reaction.

To clarify the relation of ATP hydrolysis to the DNase reaction, we have been studying the action of the *recBC* nuclease on DNA substrates with well-defined localized perturbations or structural alterations. Cole has demonstrated that introduction of crosslinks into DNA with psoralen is considerably more lethal for *E. coli* mutants defective in repair or recombination than it is for the wild-type strains (8). Also, crosslinked DNA is an inefficient substrate *in vitro* for phage λ exonuclease, the exonuclease activity of DNA polymerase I, and other enzymes likely to be involved in recombination and repair (9). These findings prompted us to observe the behavior of the *recBC* DNase on DNA crosslinked with psoralen.

Abbreviations: Psoralen-DNA, DNA that has been crosslinked by irradiation at 360 nm in the presence of psoralen (4,5′-8-trimethyl[furano-3′,2′:6,7-coumarin]); *recBC* ATPase or *recBC* DNase, the DNA-dependent ATPase and the DNase activities catalyzed by the enzyme controlled by the *recB* and *recC* genes of *E. coli*. The DNase activity has also been referred to as exonuclease V (2).

MATERIALS AND METHODS

^{3}H-Labeled DNA from *E. coli* was prepared by the method of Lehman (10); ^{14}C-labeled fd phage DNA was prepared as described (1), and phage T7 [^{3}H]DNA was prepared as described by Richardson (11), and had a specific activity of 2780 cpm/nmol unless otherwise indicated. DNA concentrations are expressed as nucleotide residues, unless otherwise indicated. The *recBC* DNase was a glycerol gradient fraction, except that enzyme prepared from *E. coli* JC4588 (*recA56*) was a DNA–cellulose fraction (7). Pancreatic DNase and micrococcal nuclease were purchased from Worthington Biochemical Corp. *N. crassa* nuclease was a concentrated hydroxyapatite fraction (12). *E. coli* B restriction enzyme was about 70% pure, and was provided by B. Eskin. Nitrogen mustard, [*bis*-(2-chloroethyl)-amine·HCl], ethidium bromide, actinomycin D, and mitomycin C were obtained commercially; 9-aminoacridine and ICR-191-E (13) were provided by Dr. B. Ames; 4,5′-8-trimethylpsoralen (14) was the generous gift of Dr. R. S. Cole. [γ-^{32}P]ATP was prepared as described (7), and used at specific activities of 600–3000 cpm/nmol.

Conditions for recBC Enzyme Incubations. Digestions contained 50 mM glycine·NaOH (pH 9.0), 10 mM $MgCl_2$, 0.67 mM dithiothreitol, 60–90 μM ATP, and 0.33 mg of acetylated bovine serum albumin per ml (7) and were at 37°. One unit of exonuclease converts 1 nmol of duplex DNA to acid-soluble products in 30 min. Throughout digestion the products are completely nonsedimentable, but only 82% acid-soluble (7); thus, the "percent DNA degraded" is calculated by dividing the percent acid-soluble by 0.82.

DNA Crosslinking. DNA was treated with 4,5′,8-trimethylpsoralen, essentially as described by Cole (15). Reaction mixtures (0.25 ml) contained 30 mM NaCl, 20 mM KPO_4 (pH 6.8), up to 50 nmol of DNA, and psoralen added in no more than 10 μl from a stock in 50% ethanol, and were illuminated for 4 hr at 15° in a water-jacketed silica microcuvette of 1-cm path length. Two General Electric F15T8-BLB "Black Lights," with a maximum emission at 360 nm, were positioned 5.1 cm from each face of the cuvette. Glass, 7-mm thick and opaque to ultraviolet light (<300 nm), was inserted between the lamps and the cuvette. The total intensity incident upon the cuvette was 0.59 mW/cm^2, as determined by potassium ferrioxalate actinometry (16). After illumination, the mixture was dialyzed extensively against 10 mM Tris·HCl (pH 8.20)–0.5 M NaCl, then against 5 mM Tris·HCl (pH 8.20). Unless otherwise specified, DNA crosslinked in the presence of 27 molecules of psoralen per molecule of DNA was used.

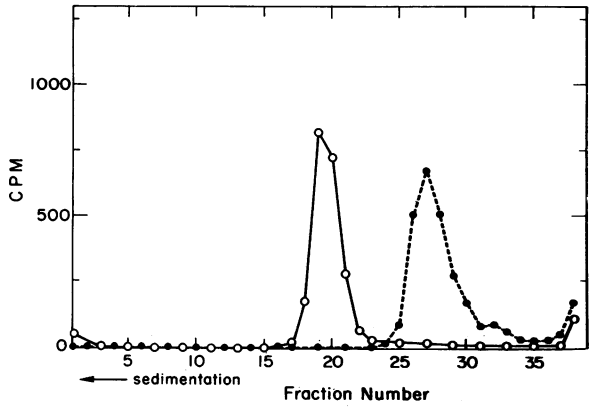

FIG. 1. Effect of psoralen treatment on sedimentation of T7 DNA in alkaline sucrose. T7 DNA (2 nmol) was irradiated at 360 nm in the presence of 1440 molecules of psoralen per DNA molecule. Another sample was exposed to psoralen in the dark. Each was layered onto a 5.2-ml linear gradient of 5–20% sucrose in 0.25 N NaOH–1 mM EDTA, then centrifuged for 60 min at 3° and 65,000 rpm in a Spinco SW65L Ti rotor. 8-Drop fractions were collected from the bottom of the tubes, neutralized with Tris·HCl, and counted by scintillation (7). The unirradiated sample sedimented exactly like DNA unexposed to psoralen. (●—●) DNA incubated in the dark; (○——○) DNA incubated under 360-nm light.

Nitrogen mustard crosslinking was performed as described by Kohn *et al.* (17). The reaction mixture (0.1 ml), containing 25 mM triethanolamine·HCl, (pH 7.20), 6 nmol of T7 DNA, and 10 nmol of nitrogen mustard, was incubated 1 hr at 37°, then dialyzed as described above. Mitomycin C crosslinking was conducted by a modification of the method of Iyer and Szybalski (18). The reaction mixture (0.1 ml) contained 30 mM KPO_4 (pH 6.50), 30 nmol of mitomycin C, and 6 nmol of T7 DNA. Freshly dissolved $Na_2S_2O_4$ (100 nmol, in H_2O), was added to initiate the reaction, then after 10 min at 37° in the dark, the mixture was dialyzed as described above.

Treatment of DNA with Intercalating Agents. E. coli DNA in 0.25 ml of 0.01 M Tris·HCl (pH 8.2) was exposed to ethidium bromide, 9-aminoacridine, ICR-191, or actinomycin D at concentrations up to one molecule of reagent per 5 DNA base pairs. These amounts approached those believed to give saturating intercalation (19), and in the case of actinomycin D exceed the amount shown by Waring to inhibit RNA polymerase by 90% (20). The agents were added as 5 μl or less from stocks in 50% ethanol, and the mixtures were incubated for 1 hr at room temperature in the dark. Changes in the emission spectra of the dyes indicative of intercalation were observed (21, 22) with a Hitachi–Perkin Elmer MPF-2A fluorescence spectrophotometer. When *E. coli* DNA was incubated with ICR-191 in 25 mM triethanolamine·HCl, (pH 7.20) at 65° for 10 min, then subjected to denaturation by heat or formamide (17), the ICR-191 fluorescence became nondialyzable, indicating covalent attachment of the compound to DNA by monofunctional alkylation.

Other Methods. The determination of ATP hydrolysis by the conversion of ^{32}P from [γ-^{32}P]ATP to a form that is nonadsorbable to Norit, the determination of DNA-nucleotide made acid-soluble, and the analysis of products of ATP hydrolysis were described (7).

RESULTS

The crosslinking of DNA with psoralen

When T7 DNA was treated with 4,5′,8-trimethylpsoralen in the presence of 360-nm light, it acquired interstrand crosslinks that remained intact during sedimentation through an alkaline sucrose gradient (Fig. 1). The crosslinked DNA sedimented about 1.5 times faster than the untreated DNA. The narrow band indicates that the treatment did not cause appreciable breakage or aggregation of the DNA. On the other hand, DNA incubated with psoralen in the dark, or DNA illuminated in the absence of psoralen, cosedimented with untreated DNA.

An upper limit could be imposed on the number of crosslinks per molecule by adjusting the amount of psoralen. T7 DNA incubated with 27–1440 molecules of psoralen per DNA molecule sedimented entirely as crosslinked material; however, at a ratio of 18, only 60% of the DNA molecules became crosslinked. If we assume that crosslinking is a random event obeying a Poisson distribution (17), about 4.4% of the psoralen molecules formed crosslinks.

Effect of *recBC* DNase on psoralen-treated DNA

When T7 psoralen-DNA was treated with up to 37 molecules of *recBC* DNase per DNA molecule, no significant change in its sedimentation in alkali was detected. This amount of nuclease degraded noncrosslinked DNA and DNA illuminated without psoralen entirely to nonsedimentable material. Al-

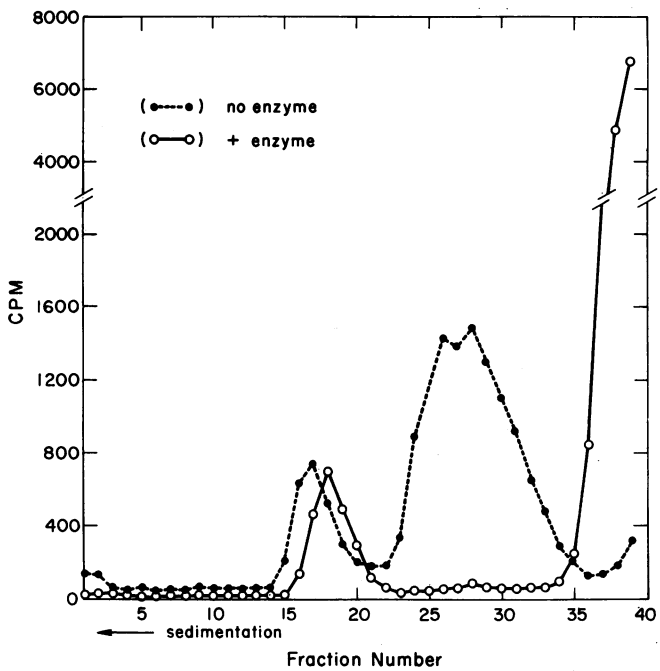

FIG. 2. Selective degradation of noncrosslinked DNA by *recBC* DNase. T7 psoralen-[^{3}H]DNA (2.25 nmol, 2780 cpm/nmol) and noncrosslinked T7 [^{3}H]DNA (0.65 nmol, 27,000 cpm/nmol) were incubated with 6 units of *recBC* DNase for 30 min as described in *Methods*. In a control reaction enzyme was omitted. Reactions were terminated by the addition of 30 μl of 0.2 M EDTA, then each mixture was layered onto alkaline sucrose gradients and analyzed as in Fig. 1, except that centrifugation was for 110 min at 50,000 rpm in a Spinco SW50.1 rotor. The apparent shift by one fraction of the heavy peak may reflect digestion of the DNA from the termini to the crosslinks.

though the psoralen-DNA appeared to be completely resistant to the DNase, the introduction of only one nick between crosslinks would not have been detected.

To test for the presence of an inhibitor in the psoralen-DNA, it was mixed with untreated DNA and exposed to the nuclease (Fig. 2). The noncrosslinked DNA was degraded completely to nonsedimentable material, while the crosslinked material remained intact. The effect of the psoralen-DNA upon the single-strand specific endonuclease activity of the enzyme (1) was also tested by observation of the degradation of single-stranded circular DNA from phage fd. [In this case, the rate-limiting step in the degradation is the first endonucleolytic break (1)]. The fd DNA was degraded in the presence of psoralen-DNA (Table 1); however, the rate of degradation was reduced to about the same extent as observed with the addition of noncrosslinked duplex DNA. Evidently, the psoralen-DNA competes with fd DNA for the *recBC* nuclease as effectively as untreated native DNA, even though it is not degraded by the enzyme.

The susceptibility of psoralen-DNA to digestion by other nucleases was also investigated. Pancreatic and micrococcal nucleases each converted psoralen-DNA entirely to nonsedimentable material, and the *E. coli* B restriction endonuclease (23) gave rise to products identical in size to those obtained from noncrosslinked DNA. On the other hand, psoralen-DNA was no more sensitive than noncrosslinked DNA to the *N. crassa* nuclease, which has a high specificity for unordered DNA (12). By these criteria, psoralen-DNA does not have gross distortions of its native structure.

Effect of psoralen-treated DNA on *recBC* ATPase

Although psoralen-DNA was not degraded by the *recBC* nuclease, it promoted the DNA-dependent ATPase activity of the enzyme at a rate slightly greater than that observed with native DNA (Fig. 3). This reaction continued as long as ATP was available. By contrast, with native DNA as cofactor, ATP hydrolysis terminated when the DNA had been digested to a limit. Identical results are observed if enzyme is prepared from a strain of *E. coli* carrying the *recA56* allele. When the products of ATP hydrolysis were analyzed, only ADP and Pi were observed, regardless of whether the cofactor was psoralen-DNA or native DNA.

TABLE 1. *Effect of psoralen-treated DNA on digestion of fd DNA*

DNA in reaction	nmol of DNA made acid-soluble	
	^{3}H	^{14}C
T7 [^{3}H]DNA	1.85	—
fd [^{14}C]DNA	—	0.85
T7 psoralen-[^{3}H]DNA	<0.01	—
fd [^{14}C]DNA plus T7 [^{3}H]DNA	0.63	0.59
fd [^{14}C]DNA plus T7 psoralen-[^{3}H]DNA	<0.01	0.40

Reaction mixtures (150 μl) were as described in *Methods*, except that they contained 90 μM ATP, 2.7 units of *recBC* nuclease, and, where indicated, 2.7 nmol of T7 psoralen-[^{3}H]DNA or untreated T7 [^{3}H]DNA (2780 cpm/nmol), or fd [^{14}C]DNA (3050 cpm/nmol). After 30 min at 37°, acid-soluble material was determined.

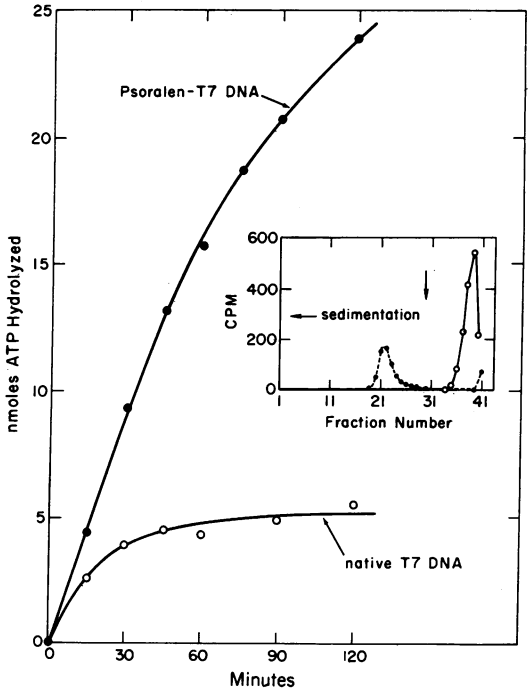

FIG. 3. Uncoupling of *recBC* ATPase from DNase with psoralen-treated DNA. Reactions containing either T7 psoralen-[^{3}H]DNA (1.95 nmol) or noncrosslinked T7 [^{3}H]DNA (2.0 nmol) were incubated with 6 units of *recBC* DNase and [γ-^{32}P]ATP. Samples (10 μl) were taken at 15-min intervals to determine the ATP hydrolyzed, and sufficient ATP was added at 15-min intervals in order to maintain the ATP concentration near 60 μM. After 120 min, 50-μl samples of each reaction were mixed with 60 μl of 0.2 M EDTA, layered onto alkaline sucrose gradients, and centrifuged and collected as described in Fig. 1. The *inset* shows the ^{3}H sedimentation profiles. (●——●) psoralen DNA; (○——○) noncrosslinked DNA. The *arrow* shows the position of sedimentation of nondegraded, noncrosslinked T7 DNA.

To test whether the psoralen-DNA must be intact to promote ATPase activity, a pancreatic DNase digest of psoralen-DNA was tested as cofactor. Only a small amount of ATP hydrolysis, which soon terminated, was observed (Fig. 4*a*). Furthermore, the digested DNA does not inhibit ATPase when added with intact psoralen-DNA. When pancreatic DNase was added after a reaction with psoralen-DNA had been initiated, ATP hydrolysis was markedly reduced, although not abolished (Fig. 4*b*). These results suggest that the psoralen-DNA must be somewhat intact in order to promote the ATPase reaction. Furthermore, the *recBC* DNase may, to a limited extent, protect the psoralen-DNA from complete digestion by pancreatic DNase.

Effect of other intercalating and crosslinking agents

Studies of the mechanism by which psoralen forms crosslinks suggest that the psoralen must intercalate between base pairs (14). Hence, the effect of psoralen on the action of the *recBC* DNase could be due simply to intercalation of the psoralen. However, DNA reacted with psoralen in the dark, or DNA mixed with either irradiated or unirradiated psoralen, was digested by the *recBC* DNase to the same extent as was untreated DNA. Furthermore, the same result was obtained for *E. coli* DNA treated with the intercalating agents, ethidium bromide, 9-aminoacridine, or actinomycin D. Duplex DNA

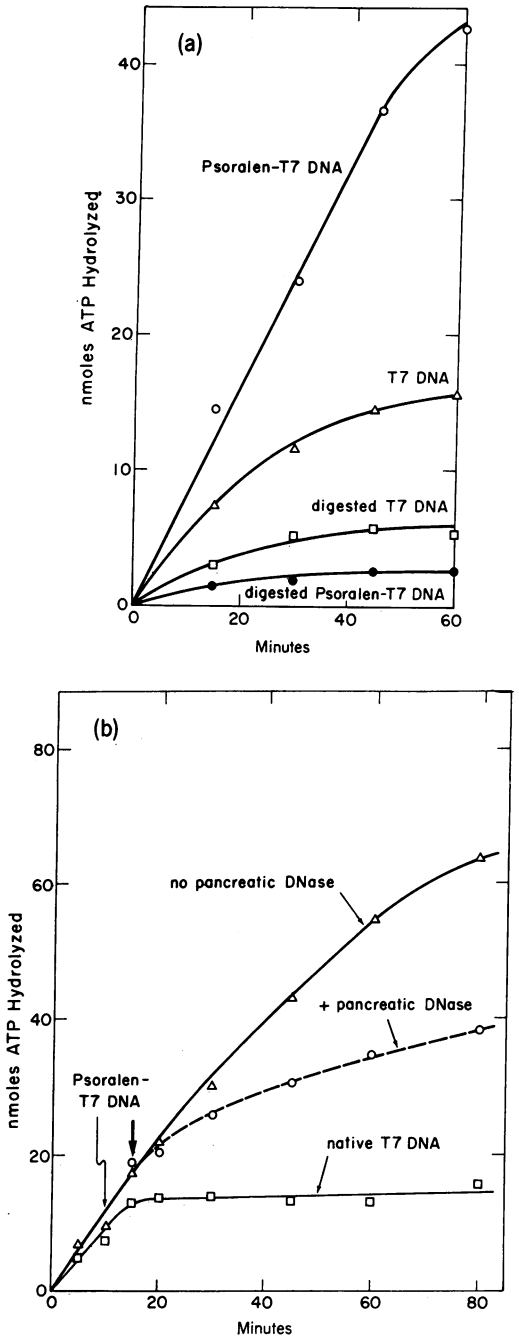

FIG. 4. (*a*) Efficacy of pancreatic DNase digests of noncrosslinked or psoralen-DNA as cofactor for *recBC* ATPase. T7 psoralen-[^{3}H]DNA (1.95 nmol) or noncrosslinked T7 [^{3}H]DNA (2 nmol) were digested with 20 μg of pancreatic DNase in 30 mM Tris·HCl (pH 8.2)–3 mM $MgCl_2$–5 mM 2-mercaptoethanol for 1 hr at 37°, then the reactions were terminated by heating at 100° for 10 min. Controls with no added pancreatic DNase were subjected to the same procedure. The digests of noncrosslinked and psoralen-DNA were 92.4% and 86.6% acid-soluble, respectively, whereas the respective control samples were 2.60% and 1.74% acid-soluble. ATPase activity was observed by incubation of 1.6 nmol of each DNA with [γ-^{32}P]ATP and 6 units of *recBC* enzyme. Samples (10 μl) were taken at 15-min intervals to determine the ATP hydrolyzed, and sufficient ATP was added at 10-min intervals in order to maintain the ATP concentration near 100 μM. In a similar experiment where a 2-fold excess of a pancreatic digest of psoralen-DNA was present during an ATPase reaction mediated by psoralen-DNA, no difference in rate from that with intact psoralen-DNA alone was observed. Hence, the pancreatic digest does not inhibit the ATPase. (*b*) Effect of pancreatic DNase addition on the *recBC* ATPase reaction. Psoralen-T7 DNA (1.95 nmol) was incubated with [γ-^{32}P]ATP and 6 units of *recBC* DNase. In another reaction, noncrosslinked DNA served as a control. At 15 min (*heavy arrow*), two 50-μl samples were withdrawn from the psoralen-DNA reaction and 20 μg of pancreatic DNase was added to one, while diluent was added to the other; then incubation was continued at 37°. Samples (10 μl) were taken at the times indicated to determine the ATP hydrolyzed, and sufficient ATP was added at 10-min intervals in order to maintain the ATP concentration near 100 μM. Values shown are corrected to refer to the entire initial reaction mixtures.

with monofunctional adducts of ICR-191 was digested by the *recBC* nuclease to a somewhat lesser extent than untreated DNA. However, the ICR-191-treated DNA behaved identically to untreated DNA as a cofactor for the *recBC* ATPase. Hence, it is unlikely that a monofunctional attachment of psoralen to DNA could be responsible for the uncoupling of the *recBC* ATPase and DNase.

Nitrogen mustard and mitomycin C, agents known to introduce interstrand crosslinks in DNA, were also used to prepare substrates for the *recBC* nuclease. Nitrogen mustard treatment resulted in a preparation that was at least 80% crosslinked, but somewhat nicked, as judged by sedimentation in alkaline sucrose. Only 7% of this DNA was degraded by the *recBC* nuclease, and ATPase continued as with psoralen DNA (Fig. 5). It was difficult to crosslink T7 DNA with mitomycin C without extensive strand breakage. However, in one preparation at least 30% of the DNA sedimented more rapidly than untreated DNA in alkali. (The remaining material was severely broken.) This DNA was only 76% degraded by the *recBC* nuclease, and was also a more efficient cofactor for the ATPase than untreated DNA (Fig. 5). These results indicate that the uncoupling of ATPase from the nuclease is a consequence of DNA crosslinking. In addition, it is clear that psoralen is a far more desirable crosslinking reagent than nitrogen mustard or mitomycin C.

DISCUSSION

Two DNA-dependent ATPase activities that have no apparent effect on the DNA have been found in *E. coli* (24). A third DNA-dependent ATPase is associated with the restriction endonuclease, but it can catalyze ATP hydrolysis for at least an hour after nuclease activity has ceased (23). The *recBC* ATPase in the presence of crosslinked DNA is a fourth DNA-dependent activity which does not detectably affect the DNA during ATP hydrolysis.

The *recBC* nuclease degrades duplex DNA to oligonucleotides of chain length 3–8 (7); we have also observed that it can degrade DNA that contains pyrimidine dimers (unpublished observation). Hence, one might have supposed that it could bypass DNA crosslinks during DNA degradation. The resistance of crosslinked DNA might then support the model of Wright *et al.* (2) that the enzyme first degrades one strand exonucleolytically, then degrades the residual single strand by the endonuclease activity that is specific for single-stranded DNA (1). Alternatively, the enzyme might degrade both strands simultaneously, but only if the strands are separable.

The uncoupling of the ATPase from DNA degradation suggests that ATP hydrolysis is not an integral part of the nuclease mechanism, but that it is a manifestation of another

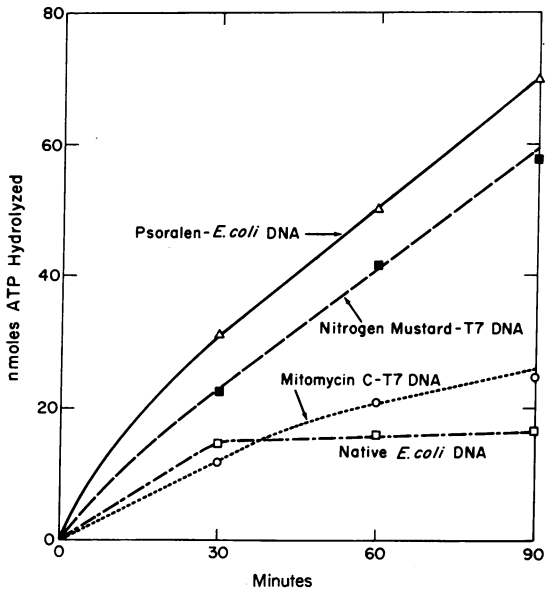

FIG. 5. ATPase activity with various crosslinked DNAs. *E. coli* [^{3}H]DNA was crosslinked with 0.45 pmol of psoralen per nmol of DNA nucleotide (the wide range of fragment sizes of this DNA precluded estimation of the maximum possible number of crosslinks per molecule). T7 [^{3}H]DNA was crosslinked with nitrogen mustard or mitomycin C. DNA (1.16 nmol) was incubated with [γ-^{32}P]ATP and 6 units of *recBC* enzyme in 0.1 ml. Samples (10 μl) were taken at 30-min intervals to determine the ATP hydrolyzed, and ATP (10 nmol) was added at 10-min intervals to maintain the ATP concentration. An identical set of reactions with unlabeled ATP in place of [γ-^{32}P]ATP was incubated at 37° for 30 min and assayed for acid-soluble radioactivity. The extents of digestion were: noncrosslinked DNA, 100%; psoralen-DNA, 15.3%; nitrogen mustard-treated DNA, 7.3%; mitomycin C-treated DNA, 75.6%.

function. Two alternative functions proposed (25) are unwinding of the double helix, or "tracking" of the enzyme along one strand of the duplex, while degrading the other. The psoralen-DNA experiments would be consistent with either model: if the ATPase reflects an enzyme-mediated configurational change* required for degradation, the enzyme might degrade up to a crosslink, then stall, attempting to form the required DNA structure. In fact, we have observed that DNA with few crosslinks is degraded to an extent consistent with the enzyme digesting from the termini to the first crosslinks. Alternatively, if the ATPase reflects a "tracking" reaction that is independent of the nuclease, but which requires lengths of intact DNA, a crosslink, by blocking degradation, would allow the DNA to serve as an ATPase cofactor indefinitely. Free ends would be required to initiate the ATPase-dependent tracking since nicked- or closed-circular duplex DNA fails to serve as an ATPase cofactor (7). These possibilities are being explored by the use of DNA with labeled termini to determine the nature of psoralen-DNA ends before and after exposure to the enzyme.

* The required configurational change would not likely be simple strand separation, however, since ATP is required, and hydrolyzed with the same efficiency, during degradation of single-stranded or duplex DNA.

Since psoralen appears to crosslink DNA at thymine residues (14), whereas nitrogen mustard and mitomycin C react at guanine residues (19), the observed behavior of the enzyme does not seem to result from a particular crosslink structure. Therefore, it might be reasonable to speculate on the existence of temporary crosslinking devices that would control the *recBC* nuclease activity. The *recA* gene product, known to limit the action of the *recBC* nuclease *in vivo* (26), might function in this manner. However, the question remains of how ATP hydrolysis would be terminated. The same question exists for the *E. coli* B restriction ATPase (23).

Finally, these experiments reaffirm the observations by Cole (15) regarding advantages of psoralen as a DNA crosslinking reagent. By enzymatic as well as physical criteria, we have found no evidence for breakage, aggregation, or gross distortion of duplex structure. In addition, the crosslinking reaction is easily controlled by variation of psoralen and DNA concentrations, intensity of illumination, or reaction time.

We are indebted to Ronald Cole and Bruce Ames for supplying materials and to Barnet Eskin for conducting the restriction enzyme reactions. A. E. K. is a postdoctoral fellow supported by Grant CA52887 from the National Cancer Institute. The research was supported by Contract no. AT(04-3)-34 from the United States Atomic Energy Commission and Grant GM19020 from the United States Public Health Service.

1. Goldmark, P. J. & Linn, S. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 434–441.
2. Wright, M., Buttin, G. & Hurwitz, J. (1971) *J. Biol. Chem.* **246**, 6543–6555.
3. Anai, M., Hirahashi, T., Yamanaka, M. & Takagi, Y. (1970) *J. Biol. Chem.* **245**, 775–780.
4. Smith, H. O. & Friedman, E. A. (1972) *J. Biol. Chem.* **247**, 2854–2858.
5. Vovis, G. F. & Buttin, G. (1970) *Biochim. Biophys. Acta* **224**, 29–41.
6. Winder, F. G. & Lavin, M. (1969) *Biochem. J.* **115**, 6P–7P.
7. Goldmark, P. J. & Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860.
8. Cole, R. S. (1971) *J. Bacteriol.* **107**, 846–852.
9. Pricer, W. E., Jr. & Weissbach, A. (1965) *Biochemistry* **4**, 200–205.
10. Lehman, I. R. (1960) *J. Biol. Chem.* **235**, 1479–1487.
11. Richardson, C. C. (1966) *J. Mol. Biol.* **15**, 49–61.
12. Linn, S. & Lehman, I. R. (1965) *J. Biol. Chem.* **240**, 1287–1293.
13. Freese, E. (1971) in *Chemical Mutagens*, ed. Hollaender, A. (Plenum Press, New York), Vol. 1, p. 50.
14. Cole, R. S. (1970) *Biochim. Biophys. Acta* **217**, 30–39.
15. Cole, R. S. (1970) *Biochim. Biophys. Acta* **224**, 660–662.
16. Hatchard, C. G. & Parker, C. A. (1956) *Proc. Roy. Soc. London* **A235**, 518–536.
17. Kohn, K. W., Spears, C. L. & Doty, P. (1966) *J. Mol. Biol.* **19**, 266–288.
18. Iyer, V. N. & Szybalski, W. (1964) *Science* **145**, 55–58.
19. Waring, M. J. (1966) in *Biochemical Studies of Antimicrobial Drugs* (16th Symposium of the Society for General Microbiology) (Cambridge University Press, London), pp. 235–265.
20. Waring, M. J. (1965) *Mol. Pharmacol.* **1**, 1–13.
21. Udenfriend, S. (1969) in *Fluorescence Assay in Biology and Medicine* (Academic Press, New York), Vol. 2, pp. 386–390.
22. Boyle, R. E., Nelson, S. S., Dollish, F. R. & Olsen, M. J. (1962) *Arch. Biochem. Biophys.* **96**, 47–50.
23. Eskin, B. & Linn, S. *J. Biol. Chem.*, in press.
24. Ebisuzaki, K., Behme, M. T., Senior, C., Shannon, D. & Dunn, D. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 515–519.
25. Winder, F. G. (1972) *Nature New Biol.* **236**, 75–76.
26. Clark, A. J. (1971) *Annu. Rev. Microbiol.* **25**, 437.