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The noncovalent complex between DNA and the bifunctional intercalator ditercalinium is a substrate for the UvrABC endonuclease of *Escherichia coli*

(bifunctional intercalator/DNA repair/UV endonuclease/antitumor agents)

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ABSTRACT **We have demonstrated that the noncovalent complex formed between DNA and an antitumor bifunctional intercalator, ditercalinium, is recognized *in vitro* as bulky covalent DNA lesions by the purified *Escherichia coli* UvrABC endonuclease. It was established that no covalent drug–DNA adduct was formed during the incubation of the drug with DNA or during subsequent incubation with the UvrAB proteins. The nucleoprotein–ditercalinium complexes appear different from those generated by repair of pyrimidine dimers. The UvrA protein is able to form a stable complex with ditercalinium–intercalated DNA in the presence of ATP, whereas both UvrA and UvrB proteins are required to form a stable complex with pyrimidine dimer-containing DNA. The apparent half-life of the UvrA– and UvrAB–ditercalinium–DNA complexes following removal of free ditercalinium is 5 min. However, if the free ditercalinium concentration is maintained to allow the intercalation of one molecule of ditercalinium per 3000 base pairs, the half-life of the UvrA– or UvrAB–ditercalinium–DNA complex is 50 min, comparable to that of the complex of UvrAB proteins formed with pyrimidine dimer-containing DNA. UvrABC endonuclease incises ditercalinium–intercalated DNA as efficiently as pyrimidine dimer-containing DNA. However, unlike repair of pyrimidine dimers, the incision reaction is strongly favored by the supercoiling of the DNA substrate. Because UvrA– or UvrAB–ditercalinium–DNA complexes can be formed with relaxed DNA without leading to a subsequent incision reaction, these apparently dead-end nucleoprotein complexes may become lesions in themselves resulting in the cytotoxicity of ditercalinium. Our results show that binding of excision repair proteins to a noncovalent DNA–ligand complex may lead to cell toxicity.**

Nucleotide excision repair of DNA damage, induced by various chemical agents or by UV irradiation, is initiated in *Escherichia coli* by an endonuclease coded for by the *uvrA*, *uvrB*, and *uvrC* genes (1, 2). The *E. coli* UvrABC endonuclease has been highly purified and characterized biochemically (1–7). In the presence of MgATP, UvrA and UvrB proteins form a high-affinity complex specifically with damaged DNA (2, 7). UvrC protein binds to the UvrAB–DNA complex to form the UvrABC endonuclease that incises the altered DNA strand (2). One incision occurs on the 5′ side and a second incision occurs on the 3′ side of the lesion. The incisions produce a lesion-containing DNA fragment, 12 or 13 bases long, that can be released later by the action of DNA helicase II and DNA polymerase I (8). The UvrABC endonuclease is believed to recognize the DNA structural alteration induced by the formation of a covalent adduct rather than the adduct itself (1). In contrast to the repair of a covalent adduct, the excision and resynthesis of a DNA fragment at the site of a noncovalent drug–DNA complex would be ineffective and futile since a new drug–DNA complex would reform immediately at a second site if the drug is not removed. In fact, response of the nucleotide excision repair system to noncovalent DNA binding drugs has not yet been observed (9). However, recent analyses of the cytotoxicity of the antitumor drug ditercalinium (10, 11) have suggested that, indeed, a drug–DNA reversible complex might be recognized by the nucleotide excision repair of *E. coli* (12). Ditercalinium (structure in Fig. 1) is a *7H*-pyridocarbazole dimer that bisintercalates into the DNA through the major groove (13). It forms a high-affinity reversible complex with DNA. In *E. coli*, ditercalinium was cytotoxic only to a *polA* strain and to a *lig 7* strain at nonpermissive temperature (12). The suppression of ditercalinium cytotoxicity in *polA* strains by the *uvrA* mutation suggested the involvement of the nucleotide excision repair process in the mechanism of cytotoxicity (12). It was further suggested that ditercalinium lured the repair system into an abortive and futile DNA repair attempt by inducing a DNA structural alteration mimicking that of bulky covalent adducts (12). In this paper we present evidence that, indeed, the reversible ditercalinium–DNA complex is recognized *in vitro* as a lesion by the UvrABC endonuclease. Unusual and unexpected properties of the nucleotide excision repair system are revealed by this study.

MATERIAL AND METHODS

UvrABC Endonuclease. The *E. coli* UvrA, UvrB, and UvrC proteins used in these studies were purified as described (6) to >95% purity as estimated using SDS/polyacrylamide gel electrophoresis.

Preparation of pSP65 Plasmid DNA. *E. coli* ATY 0404 [(*recA* HF4704, pSP65 (Promega, 3005 base pairs), amp^R)] was grown in the presence of 100 μg of ampicillin per ml in TPA medium for ^{3}H labeling of pSP65 DNA (2) or in Luria broth medium for preparation of nonradiolabeled DNA. The supercoiled covalently closed circular form (F-I) of pSP65 plasmid DNA was purified as described (2).

Relaxation of Plasmid DNA. The plasmid DNA was relaxed in the UvrABC endonuclease reaction buffer (without ATP, with 20% glycerol), in a 50-μl reaction volume. Six micrograms of pSP65 DNA was treated with 8 units of wheat germ

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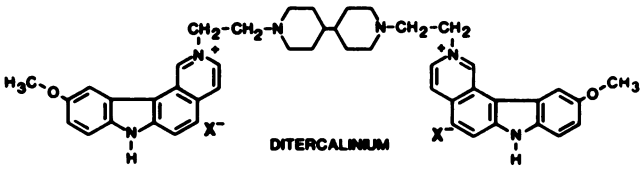

FIG. 1. Structure of ditercalinium. X = tetramesylate.

topoisomerase I (Promega) or with rat liver topoisomerase I (gift from G. Muzard) for 30 min at 37°C. After two phenol extractions, the relaxed form of DNA (F-II) was ethanol precipitated (14), resuspended in 20 μl of 10 mM Tris·HCl/0.4 mM EDTA, pH 7.6, microdialyzed using a Millipore VSWP 2500 membrane (15) for 90 min at room temperature, and analyzed on 1% agarose gels.

Ditercalinium Treatment of Plasmid DNA. A saturated stock solution of ditercalinium (Roger Bellon Laboratory, Neuilly-sur-Seine, France), at about 1 mM in water, was made fresh and filter sterilized. The concentration of ditercalinium was determined by using a molar extinction coefficient of 65,000 at 262 nm. Ditercalinium was diluted in water in siliconized tubes just prior to the incubation with DNA.

UvrABC Protein–DNA Complex Filter Binding Assay. The formation of stable UvrAB protein–DNA complexes was measured by trapping the native protein–DNA complexes on nitrocellulose filters as described (2). Briefly, the 140-μl reaction mixture consisted of 40 mM potassium morpholinopropanesulfonate buffer at pH 7.6, 85 mM KCl, 1 mM dithiothreitol, 15 mM $MgSO_4$, 2 mM ATP, 200 fmol (each) of UvrA and UvrB, and 17 fmol of [^{3}H]DNA preincubated with ditercalinium or irradiated by UV at 254 nm as described by Yeung *et al.* (2). The mixture was incubated at 37°C for 10 min, and the reaction was terminated by the addition of 5 ml of cold 2× SSC (0.3 M NaCl/0.03 M trisodium citrate). The DNA molecules bound to protein were collected by trapping onto a BA 85 nitrocellulose filter.

UvrABC Endonuclease Assay. The UvrABC endonuclease nicking of ditercalinium-intercalated DNA or UV-irradiated DNA was measured as the conversion of F-I to F-II DNA by UvrABC endonuclease in a nitrocellulose filter binding assay previously described in detail (2, 16). The assay uses 17 fmol of ^{3}H-labeled F-I DNA (133,000 cpm/μg) prepared as described (2) and scores for the first nick in the F-I DNA. For assay with unlabeled DNA, analyses by 1% agarose gel were used to follow the conversion of F-I to F-II DNA by the UvrABC endonuclease. The reaction mixture, 560 μl, contained 68 fmol of DNA and 600 fmol of each Uvr protein. After 10 min of incubation at 37°C, SDS was added (0.5% final concentration). Then proteinase K was added (100 μg/ml final concentration), and the samples were incubated at 50°C for 60 min. The samples were phenol extracted, precipitated with isopropyl alcohol (14), redissolved in 20 μl of 10 mM Tris·HCl/0.4 mM EDTA, pH 7.6, and microdialyzed (see above) before electrophoresis.

RESULTS

UvrA or UvrAB Recognition of the Ditercalinium–DNA Complexes. The result of the experiment presented in Fig. 2 shows that the amounts of UvrA or UvrAB forming complexes with ditercalinium–intercalated DNA depend on the ditercalinium concentration. The effect of the number of lesions in each plasmid DNA on its binding by UvrAB is similar for pyrimidine dimers and ditercalinium intercalation. As few as one or two lesions per 3000-base-pair plasmid are sufficient for the protein–DNA complexes to bind to the nitrocellulose filter. UvrA protein, independent of the UvrB protein, binds to ditercalinium–intercalated DNA with an affinity equal to that of the UvrAB–ditercalinium–DNA or

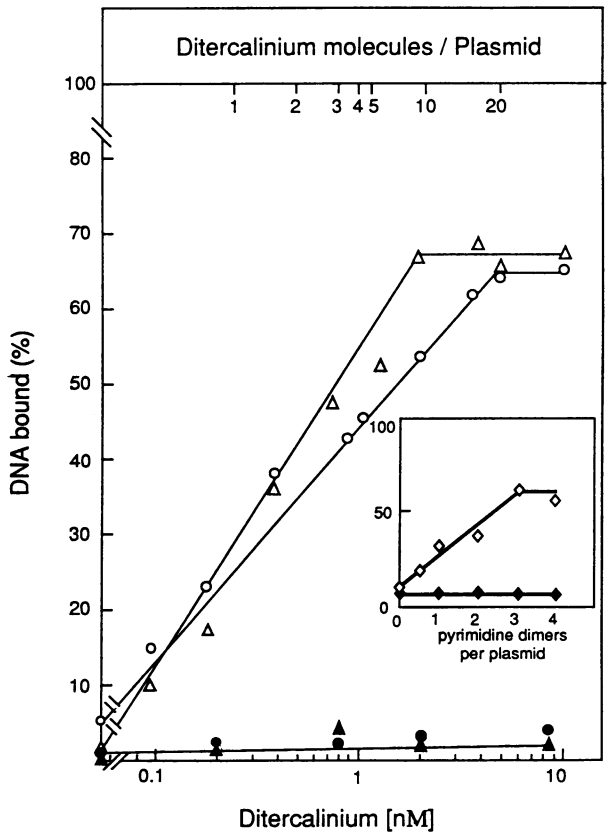

FIG. 2. Effect of ditercalinium concentration on the formation of the UvrA– and UvrAB–ditercalinium–DNA complexes. DNA was incubated with ditercalinium in the UvrABC endonuclease assay buffer. Fourteen microliters of ditercalinium solution (1–77.1 nM) was added to 26 μl of buffer containing 30 ng of ^{3}H-labeled pSP65 F-I DNA to produce the final concentrations shown. After 60 min of incubation at room temperature in the dark, Uvr proteins were added. The final volume of the reaction mixture was 140 μl and contained 17 fmol of labeled DNA, 200 fmol of each Uvr protein, and ditercalinium (0.1–7.71 nM). After 10 min of incubation at 37°C, the Uvr protein–DNA complex filter binding assay was performed. The number of intercalated molecules of ditercalinium per plasmid has been calculated according to the equation of Le Pecq *et al.* (17). The ionic strength and the pH of the UvrABC endonuclease buffer are close to those of the buffer used in the ditercalinium binding studies (10). Thus, the same ditercalinium dissociation constant ($K_d = 10^{-7}$ M) was used in the calculation. UvrA (△, ▲) or UvrAB (○, ●) binding was obtained, respectively, in the presence or in the absence of ATP. (*Inset*) UvrAB binding on UV-irradiated DNA containing increasing amounts of pyrimidine dimer in the presence (◇) or in the absence (◆) of ATP.

the UvrAB–UV-irradiated DNA complexes. The binding of UvrA or UvrAB to ditercalinium–intercalated DNA is dependent on the presence of MgATP as observed in the case of UvrAB binding to UV-irradiated DNA (Fig. 2 *Inset*).

Interaction of the UvrAB Proteins with the Ditercalinium–DNA Complex Does Not Involve the Covalent Binding of the Drug to the DNA. We tested for the possibility of covalent adduct formation on DNA by the incubation of ditercalinium with DNA and the subsequent incubation of the ditercalinium–DNA complexes with UvrAB proteins under the UvrABC endonuclease assay conditions. The experiment described in Table 1 shows that after phenol extraction of the UvrAB–ditercalinium–DNA complex no covalent lesions are created involving the DNA as diagnosed by the inability of UvrAB to bind to this DNA.

Stability of the UvrA– or UvrAB–Ditercalinium–DNA Complexes. The study of the stability of the UvrA– or UvrAB–ditercalinium–DNA complexes is complicated by the fact

Table 1. Absence of covalent DNA modification during ditercalinium–DNA incubation

	% DNA bound*	
	Before phenol extraction	After phenol extraction
DNA	9	8.4
+ ditercalinium	75	8.2
+ UV	64	59

In a 1.4-ml reaction volume, a mixture of 170 fmol of ^{3}H-labeled pSP65 DNA and 2 pmol of UvrAB was incubated for 10 min at 37°C. Part of the reaction mixture was used to ascertain UvrAB–ditercalinium–DNA complex formation by the filter binding assay. The rest was extracted twice with phenol and the DNA was ethanol precipitated. The purified DNA was suspended in the UvrABC endonuclease buffer, and the protein–DNA filter binding assay, in the presence of fresh UvrA and UvrB proteins, was performed to test for the possibility of bulky-adduct formation from the previous incubation. UV-irradiated DNA (three pyrimidine dimers per molecule of plasmid) and nontreated DNA were used as controls. Results are expressed as the percentage of the total amount of ^{3}H-labeled pSP65 DNA used in the assay.
*% of the total DNA concentration in the assay.

that ditercalinium is not covalently bound to DNA (above). In the absence of proteins, the relaxation time for ditercalinium release from the DNA is ≈1 sec (13). Several experimental conditions were designed to measure the rate of release of the individual components of the UvrA– or UvrAB–ditercalinium–DNA complexes (Table 2). When the loss of ditercalinium from the complex is prevented by including ditercalinium in the dilution medium to maintain the free ditercalinium concentration (lines 7 and 8), the dissociation rate of the UvrAB–DNA complex upon dilution is long

Table 2. Half-lives of decay of the Uvr protein–DNA complexes

			Protein–DNA complex half-life decay	
Uvr protein	Treatment of pSP65 DNA	Dilution buffer	Temperature, °C	Time, min
1. UvrAB	UV	Chelator	4	65
2. UvrAB	Dit	Chelator	4	5
3. UvrAB	UV	MgATP	37	30
4. UvrAB	Dit	MgATP	37	5
5. UvrAB	UV	MgATP + DNA	37	30
6. UvrAB	Dit	MgATP + DNA	37	5
7. UvrAB	Dit	MgATP + Dit + DNA	37	50
8. UvrAB	Dit	MgATP + Dit	37	55
9. UvrA	UV	Chelator	4	(5)*
10. UvrA	Dit	Chelator	4	5
11. UvrA	Dit	MgATP	37	6
12. UvrA	Dit	MgATP + DNA	37	5
13. UvrA	Dit	MgATP + Dit + DNA	37	50
14. UvrA	Dit	MgATP + Dit	37	55

The binding of UvrA, UvrB protein combination to DNA was assessed with the protein–DNA filter binding assay. Ditercalinium (Dit) concentration was 5.08 nM and the UV-irradiated DNA contained three pyrimidine dimers per molecule of plasmid. The assay mixture consisted of 17 fmol of ^{3}H-labeled pSP65 DNA and 200 fmol of each of the respective Uvr proteins incubated for 10 min at 37°C. The binding was terminated by dilution of the reaction mixture with 5 ml of either 2× SSC (chelator) or the reaction buffer with or without unlabeled DNA with or without ditercalinium as indicated. In these dilution buffers, the nonlabeled DNA and ditercalinium concentrations were the same as those used in the binding assay. Samples were then incubated at the temperatures indicated. The stability of the nucleoprotein complexes is expressed as the half-life of decay of the amount of ^{3}H-labeled pSP65 DNA retained by the nitrocellulose filter.
*Seconds.

(about 50 min) and of the same order as in the case of UV-irradiated DNA (lines 3 and 5). However, contrary to what is observed with UV-irradiated DNA, the UvrA–ditercalinium–DNA complex (lines 13 and 14) is as stable as the UvrAB–ditercalinium–DNA complex (lines 7 and 8). When both free ditercalinium and Uvr proteins concentrations are decreased upon dilution (lines 2, 4, 10, and 11), the observed rates of dissociation are shortened to 5–6 min. The half-lives of decay are almost identical whether the dilution buffers contain unlabeled competing DNA or not. These results show that the UvrA or UvrAB proteins dissociate from the ditercalinium–DNA complex at the same rate as the dissociation of UvrAB proteins from UV-irradiated DNA. Moreover, ditercalinium can dissociate from the overall complex much faster than the Uvr proteins but much slower than ditercalinium alone from the DNA.

UvrABC Endonuclease Incision of Ditercalinium–Intercalated DNA. The nicking of ditercalinium–intercalated DNA by the UvrABC endonuclease is shown in Fig. 3. The UvrABC endonuclease nicking is dependent on low concentrations of ditercalinium. Maximum nicking is observed at 1 nM, which corresponds to three intercalated molecules of ditercalinium per molecule of plasmid. The efficiency of UvrABC endonuclease incision of ditercalinium–intercalated DNA is similar to the incision efficiency obtained at sites of pyrimidine dimers (Fig. 3). The conversion of supercoiled F-I DNA to relaxed F-II DNA was also analyzed by using agarose gel electrophoresis. For incision to occur on ditercalinium–intercalated DNA, the combination of UvrA, UvrB, and UvrC proteins is required as observed also for

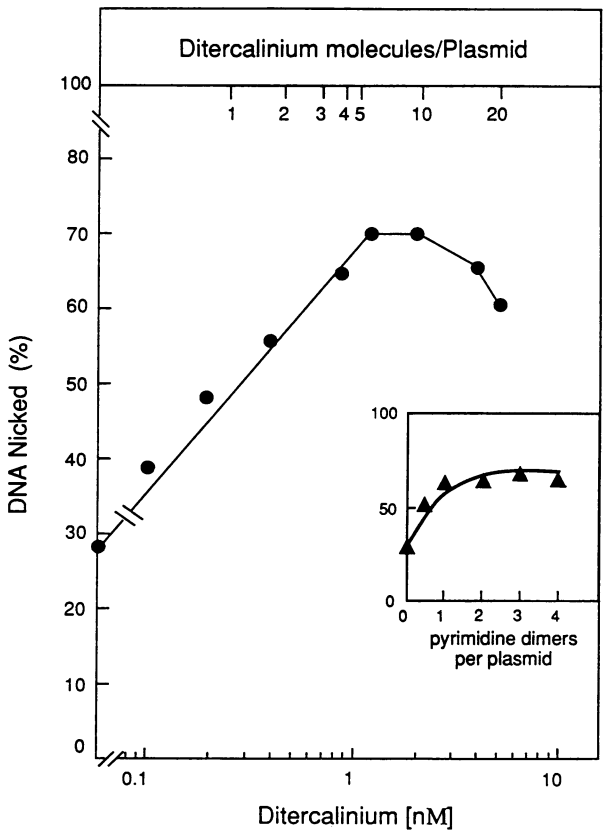

FIG. 3. Dependence of the DNA nicking by UvrABC endonuclease on the concentration of ditercalinium. ^{3}H-labeled pSP65 DNA was incubated with ditercalinium and UvrA, -B, and -C proteins as described in the legend to Fig. 2. After 10 min of incubation at 37°C, the reaction mixture was subjected to denaturation and renaturation and filtered on a nitrocellulose filter (●) as described (16). (*Inset*) Results obtained with UV-irradiated DNA (▲).

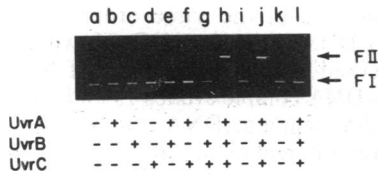

FIG. 4. Analysis by agarose gel electrophoresis of UvrABC endonuclease nicking of ditercalinium–intercalated DNA. Nonlabeled pSP65 DNA was incubated with ditercalinium at 5.08 nM. The reaction mixture volume was 560 μl and contained 68 fmol of DNA. Six hundred femtomoles each of UvrA, UvrB, and UvrC proteins was added singly or in combination, as indicated by + and − signs, to pSP65 DNA (lanes a–h). The results obtained with UV-irradiated DNA (three pyrimidine dimers per plasmid) and nonirradiated DNA are shown in lanes i–j and lanes k–l, respectively.

UV-irradiated DNA (Fig. 4). There is no incision in the absence of ATP (data not shown).

Role of the DNA Superhelicity in the Incision of Ditercalinium–Intercalated DNA by UvrABC Endonuclease. The UvrABC endonuclease nicking of supercoiled and relaxed DNA was visualized by electrophoresis in an agarose gel containing ethidium bromide. The three DNA forms—relaxed covalently closed circular DNA, relaxed circular nicked DNA, and circular supercoiled DNA—are resolved in this gel. The results are shown on Fig. 5. The presence of ditercalinium intercalation results in a more efficient incision of the supercoiled DNA by the UvrABC endonuclease than of relaxed DNA (lanes 10 and 12). For UV-irradiated DNA the relaxed and supercoiled DNA are incised with the same efficiency (lanes 1–8).

DISCUSSION

The recognition of the ditercalinium–DNA complex by the purified UvrABC endonuclease was studied *in vitro*. The results presented here prove that, indeed, a noncovalent drug–DNA complex is a substrate for the UvrABC endonuclease. Furthermore, this report broadens the range of DNA structural alterations recognized by the UvrABC endonuclease.

Our results demonstrate that a single ditercalinium molecule bound per DNA molecule is sufficient to induce the binding of the UvrAB proteins. The binding efficiencies of these proteins to the ditercalinium–DNA complex and UV-irradiated DNA are comparable. It was demonstrated that no covalent adduct was required for the recognition process, although the formation of such a product was unlikely in light

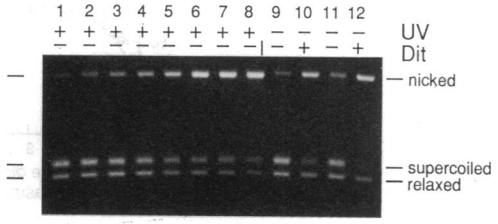

FIG. 5. UvrABC endonuclease nicking of ditercalinium–intercalated supercoiled and relaxed DNA. A mixture containing the same amount of nonlabeled supercoiled (F-I) and relaxed (F-II) pSP65 DNA was treated as described in the legend to Fig. 4. The ditercalinium (Dit) concentration was 2.09 nM; the same results were obtained with 5.08 nM. Nontreated DNA and ditercalinium–intercalated DNA were incubated with UvrABC proteins for 10 min (lanes 9 and 10) or for 20 min (lanes 11 and 12), respectively. The same experiment was performed with UV-irradiated DNA (three pyrimidine dimers per plasmid). The times of incubation with UvrABC proteins were 0, 1, 2, 3, 5, 8, 10, and 20 min in lanes 1–8, respectively. Electrophoresis was carried out in a 1% agarose gel containing 0.2 μg of ethidium bromide per ml at 15 mA for 14 hr.

of the known ditercalinium chemistry. Fully native DNA, no longer recognized by the UvrAB proteins, was obtained by phenol extraction of the UvrAB–ditercalinium–DNA complex and thus established that no covalent bond was formed. The Uvr protein complexes on ditercalinium–intercalated DNA exhibit several unexpected properties.

UvrA Forms a High-Affinity Complex with Ditercalinium–Intercalated DNA. Analyses of UvrA and UvrAB binding to ditercalinium–DNA complex reveal that UvrA forms a complex as stable as that formed by UvrAB (Fig. 2 and Table 2), whereas on UV-irradiated DNA, UvrB protein is required to stabilize the UvrA–DNA complex (2, 3).

The Stability of the UvrA–, UvrAB–Ditercalinium–DNA Complexes Depends on the Free Ditercalinium Concentration. Ditercalinium can potentially dissociate from the UvrAB–ditercalinium–DNA complex independent of the UvrA and UvrB proteins because ditercalinium is not covalently bound to DNA. When the dissociation rate of this complex is measured under conditions of constant ditercalinium concentration, the loss of ditercalinium from the complex is minimized. Under that condition, the rate of dissociation of UvrAB from the complex is similar to that of the corresponding complex with UV-irradiated DNA ($t_{1/2}$ = 55 min). When the dissociation rate of UvrAB proteins from the ditercalinium–DNA complex is measured under conditions that do not prevent the loss of the ditercalinium from the complex (no ditercalinium in the dilution buffer and/or addition of an excess of unlabeled DNA), the dissociation rate of the UvrAB proteins from the complex is much faster ($t_{1/2}$ = 5 min). Sancar and Sancar (1) proposed that the UvrABC endonuclease binds to the DNA face opposite to the one containing the lesion. Such a model would account for the observed independent dissociation of ditercalinium from its complex with DNA and Uvr proteins.

The UvrABC Endonuclease Incision of Ditercalinium–Intercalated DNA Is Favored by the Supercoiling of the DNA Substrate. In the presence of ditercalinium, supercoiled DNA is nicked more rapidly by UvrABC endonuclease than the relaxed DNA, although the UvrAB protein binding to both types of DNA is identical (data not shown). This observation is in agreement with the proposal of Oh and Grossman (18) that the DNA conformation that is recognized in UvrAB binding is not the same as the subsequent DNA conformation that is involved in UvrABC endonuclease incision. The requirement of torsional constraint on incision by the UvrABC endonuclease in the case of ditercalinium contrasts with that observed for the repair of UV-irradiated DNA and anthramycin–DNA adducts. In the former case, no effect of supercoiling was detected (Fig. 5), whereas in the latter case, it is the relaxed modified DNA that is cut more efficiently than the supercoiled DNA (19).

Physiological Consequences of the Recognition of Noncovalent Drug–DNA Complexes by the UvrABC Endonuclease. The reversible ditercalinium–DNA complex mimics a bulky DNA lesion, yet the UvrABC endonuclease is inherently unable to cope with a reversible lesion since it cannot eliminate the causative agent. In the presence of such an agent, the action of the UvrABC endonuclease, instead of being beneficial to the cell, would become damaging, as shown by the requirement of a functional *uvrA* gene for the ditercalinium cytotoxicity (12). This cytotoxicity could result from the accumulation of errors during the futile repair cycles and/or from the induction of dead-end Uvr protein–DNA complexes. These complexes may be DNA lesions in themselves that may interfere with DNA metabolism or deplete the cell of DNA repair enzymes. The mechanisms, revealed by studying the repair of bifunctional intercalator DNA lesions, may provide insights into the mechanism of lesion recognition in nucleotide excision repair as well as the antitumor mechanism of ditercalinium.

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1. Sancar, A. & Sancar, G. (1988) *Annu. Rev. Biochem.* **57,** 29–67.
2. Yeung, A. T., Mattes, W. B. & Grossman, L. (1986) *Nucleic Acids Res.* **14,** 2567–2582.
3. Seeberg, E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2569–2573.
4. Yeung, A. T., Mattes, W. B., Oh, E. Y., Yoakum, G. H. & Grossman, L. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6157–6161.
5. Sancar, A. & Rupp, W. D. (1983) *Cell* **33,** 249–260.
6. Yeung, A. T., Mattes, W. B., Oh, E. Y., Yoakum, G. H. & Grossman, L. (1986) *Nucleic Acids Res.* **14,** 8535–8556.
7. Yeung, A. T., Jones, K. B., Capraro, M. & Chu, T. (1987) *Nucleic Acids Res.* **12,** 4957–4971.
8. Caron, P. R., Kushner, S. R. & Grossman, L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4925–4929.
9. Lambert, B., Laugâa, P., Roques, B. P. & Le Pecq, J.-B. (1986) *Mutat. Res.* **166,** 243–254.
10. Pelaprat, D., Delbarre, A., Le Guen, I., Roques, B. P. & Le Pecq, J.-B. (1980) *J. Med. Chem.* **23,** 1336–1343.
11. Esnault, C., Roques, B. P., Jacquemin-Sablon, A. & Le Pecq, J.-B. (1984) *Cancer Res.* **44,** 4355–4360.
12. Lambert, B., Roques, B. P. & Le Pecq, J.-B. (1988) *Nucleic Acids Res.* **16,** 1063–1078.
13. Delbarre, A., Delepierre, M., Garbay, C., Igolen, J., Le Pecq, J.-B. & Roques, B. P. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2155–2159.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Marusyk, R. (1980) *Anal. Biochem.* **105,** 403–404.
16. Riazuddin, S. & Grossman, L. (1977) *J. Biol. Chem.* **252,** 6280–6286.
17. Le Pecq, J.-B., Le Bret, M., Barbet, J. & Roques, B. P. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2915–2919.
18. Oh, E. Y. & Grossman, L. (1986) *Nucleic Acids Res.* **14,** 8557–8571.
19. Walter, B. W., Pierce, J., Case, R. & Tang, T. (1988) *J. Mol. Biol.* **203,** 939–947.