Volume 16 Number 3 1988 Nucleic Acids Research

Induction of an abortive and futile DNA repair process in *E.coli* by the antitumor DNA bifunctional intercalator, ditercalinium: role in *pol*A in death induction

Bernard Lambert, Bernard P.Roques[1] and Jean-Bernard Le Pecq

Unité de Physicochimie Macromoléculaire, LA 147 CNRS et U 140 INSERM, Institut Gustave Roussy, 94805 Villejuif Cédex and [1]Département de Chimie Organique, UA 498 CNRS et U 226 INSERM, UER des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75006 Paris, France

Received December 4, 1987; Accepted January 8, 1988

ABSTRACT.

Ditercalinium, an antitumor bifunctional intercalator which forms a high affinity reversible complex with DNA, was found to be specifically cytotoxic for polA and lig7 E. coli strains. In the polA strain, the cytotoxic effect of ditercalinium was suppressed by the uvrA mutation. DNA single strand breaks accumulated in presence of ditercalinium at high temperature in lig7 strains but not in polA strains. Ditercalinium caused no DNA synthesis inhibition although it was able to induce SOS functions. It is proposed that the ditercalinium DNA complex because of its non covalent nature acts as a dummy lesion for the UV repair system in E. coli leading to a futile and abortive repair process.

Polymerase I appears to be required to prevent the malfunctioning of a DNA repair process triggered by molecules forming non covalent complex with DNA.

INTRODUCTION.

To obtain molecules with a high binding affinity for DNA, polyfunctional intercalators have been synthetized and studied (1,2). DNA binding agents with affinity as high as $10^{14}M^{-1}$ were obtained (3). Several bifunctional intercalators, among them ditercalinium (NSC 335153), elicit strong anticancer activity on a variety of animal tumor models (4,5). Ditercalinium (structure shown in figure 4) is derived from 7H-pyridocarbazole and binds to DNA from the major groove (6,7). The mechanism of action of ditercalinium appears totally different from that of other anticancer agents (8). One of the most intriguing of its properties is the induction of a delayed toxicity in animal cells. Cells exposed for a short time to ditercalinium continue to grow for 5 to 6 generations before dying (8).

Prior to the introduction of this new agent in clinical trials, mutagenic tests were performed in bacterial systems. This led us to isolate an E. coli mutant strain specifically sensitive to ditercalinium (9). The discovery that the mutation confering sensitivity to ditercalinium was polA led us to

study in detail the interference of this compound with the UV repair system in E. coli. We show here that the DNA-ditercalinium complex, although reversible and non covalent, is recognized by the UV repair system in E. coli.

MATERIAL AND METHODS.

Bacterial strains.

Strains used in this paper are described in Table 1 :

Bacterial construction.

Bacterial strains were constructed by P1 transduction according to Miller (20). Tn10 and Tn5 insertions confering respectively the tetracycline and the kanamycin resistance were used as selective marker. A Tn10 inserted in the gene named zig which is located at 86 min on the bacterial genome, was used as a marker of transduction of the polA gene. The zig gene is 40 % co-transductible with polA gene. polA mutants were revealed by their increased MMS sensitivity (2 mM). Double mutants, polA1 lexA(ind$^-$) polA1 uvrA, polA12 uvrA, polA12 uvrC, polA12 uvrD were revealed by their increased sensitivities to both MMS and UV. Double mutant polA1 lon was revealed both by the mucoïd character of lon strain and its high MMS sensitivity.

As it was observed that Tn10 decreased the ditercalinium sensitivity of polA mutants (data not shown), Tn10 was removed in all strains by the method of Bochner et al. (21).

Media.

LB and M9 media were made as described (20).

M9 medium was supplemented with 0.3 % casaminoacids.

LBT plates consists of LB medium plus 50 µg/ml of thymine and 12 g/l agar.

For P_1 transduction 15 µg/ml of tetracycline or 40 µg/ml of kanamycin was added to LBT plates containing 2.5 10^{-3} M sodium citrate.

Bochner medium was used to select tetracycline sensitive strains as described (21).

Chemical reagents.

The various 7H-pyridocarbazole dimers were synthetized as described previously (4,5). Ditercalinium, a 7H-pyridocarbazole dimer (NSC 335153) was from Roger Bellon Laboratory. Tetracycline hydrochloride, ONPG (ortho-nitrophenol-β-D-galactopyranoside) and calf thymus DNA were from Boehringer Mannheim. Kanamycin sulfate was from Theraplix Laboratory. Chloramphenicol was from Roussel Uclaf. MMS was from Aldrich. Fusaric acid was a gift of Dr. R. D'Ari. 3 MM filters were from Whatman. Chlortetracycline was from Sigma.

Table 1. E. coli strains used in the work

Strain	Genotype	Source, reference or construction
AB1157	F⁻, thr-1, leu-6, proA-2, his-4, thi-1 arg E_{3-} $lacY_1$, $galK_2$, ara-14, xyl-5, mtl-1, tsx-33, strA31, supE37	14
AB2500	as AB1157 also uvrA6	M. Cox
AB2499	as AB1157 also uvrB5	M. Cox
AB2498	as AB1157 also uvrC34	M. Cox
AB2463	as AB1157 also recA13	
AB2494	as AB1157 also lexA	
GW2100	as AB1157 but umuC122::Tn5, Kn^R	10
N2057	as AB1157 but ruv60::Tn10	11
AB2429	as AB1157 but uvrA37, uvrC34 ($\lambda uvrA^+ ssb^+$)	12
AB1884	as AB1157 but uvrC34 ($\lambda uvrC^+$)	13
GC4671	Δlon-100, zab300::Tn10, Tc^R	15
N2668	F⁻, lig7, relA1, spoT1, bglR6, rpsL150	B. Bachmann (16)
RH2456	polA12 derives from MM383	M. Radman and (17)
GY3926	uvrD101	P. Moreau
GC4510	sfiA11, sfiC	R. D'Ari
B1654	sfiA::Tn5, Kn^R	R. D'Ari
GC2281	lexA (ind⁻), malB::Tn9 (Cm^R, λ^S), pro, his, arg, lac, gal, str^R	R. D'Ari
GC2736	polA214, zig::Tn10, Tc^R	R. D'Ari
GC4715	F⁻, thyA, lac, str idem JG113	S. Boiteux (18)
GC4716	as GC4715 but polA1	S. Boiteux
GC4718	as GC4715 but (λp(sfiA, lacZ)CIind)	S. Boiteux
GC4727	as GC4715 but polA1, (λp(sfiA, lacZ)CIind)	S. Boiteux
SR1601	uvrA::Tn10, Tc^R	R. Sharma
BL199	pol⁺, zig::Tn10, uvrC35, Tc^R	from GC2736 and AB2498 (a)
BL200	polA1, zig::Tn10	from BL199 and GC4716 (a)
BL201	as RH2456 but polA12 zig::Tn10, Tc^R	from BL199 and RH2456 (a)
BL202	polA1 uvrA::Tn10, Tc^R	from GC4716 and SR1601 (a)
BL203	polA1, sfiA::Tn5, Kn^R	from GC4716 and B1654 (a)
BL204	polA1, umuC122::Tn5, Kn^R	from GC4716 and GW2100 (a)
BL206	polA12, zig::Tn10, uvrD101, Tc^R	from BL201 and GY3926 (a)
BL207	polA12, zig::Tn10, uvrC35, Tc^R	from BL201 and AB2498 (a)
BL208	polA1, Δlon-100 zab300::Tn10, Tc^R	from GC4716 and GC4671 (a)
BL209	polA1, zig::Tn10, sfiA11 sfiC	from BL200 and GC4510 (a)
BL300	lig7, thy⁺	from N2668 (b)
BL301	polA1, thy⁻	from GC4716 (b)
BL500	polA1, Δlon-100	from BL208 (c)
BL501	polA1, uvrA	from BL202 (c)
BL503	polA12, uvrC35	from BL207 (c)
BL504	polA12, uvrD101	from BL206 (c)
BL505	polA1 sfiA11 sfiC	from BL209 (c)
BL506	polA1 malB::Tn9 (Cr^R, λ^S)	from GC4716 and GC2281 (a)
BL507	polA1 lexA (ind⁻) malB::Tn9 (Cr^R,λ^S)	from GC4716 and GC2281 (a)

(a) P1 transduction ; (b) trimethoprim selection (20) ; (c) Tn10 removed (21)

Sucrose was from B.D.H. Plastic beakers were from Prolabo. [^{14}C]thymine (55 mCi/mmol) and [^{3}H]thymine (46 Ci/mmol) were from Amersham. [^{14}C]uracil (58 mCi/mmol) and [^{14}C] leucine (60 mCi/mmol) were from C.E.A.

Cytotoxicity measurement.

Bacteria were grown in M9 medium containing 50 μg/ml of thymine at 33°C. At a 0.4 absorbance (λ = 600 nm) bacteria were diluted fourfold. 2 ml aliquots of bacterial suspension were treated with various concentrations of the compounds and incubated at 33°C or 42°C depending on the thermosensitivity of the polA mutation. After 120 min of incubation, bacteria were plated on LBT plates. Colonies were counted after an overnight incubation at 33°C. Plastic beakers were used to minimize wall adsorption of compounds which is probably responsible for a relatively large variability of the results. Therefore measurements were repeated three times or more so that standard errors could be estimated.

SOS induction measurements.

Strains GC4727 or GC4718 were incubated and treated at 37°C as in the cytotoxicity method. After 120 min of incubation, β-galactosidase synthesis measurement was performed as described previously (22).

Selection of ditercalinium resistant polA mutant.

Strain GC 4716 (polA1) was grown in LBT medium at 33°C up to a 600 nm optical density of 0.8. Bacteria were diluted and each dilution was spread on LBT plates containing 10 μg/ml of ditercalinium. After an overnight incubation at 33°C resistant colonies were picked up and grown in LBT medium. Resistant colonies were tested for UV and MMS sensitivity.

Macromolecular synthesis kinetics.

Bacteria (polA1 sfiA mutant) were grown in M9 medium supplemented with the required amino acids and containing 10 μg/ml of thymine. Bacteria were treated or not with 0.1 μg/ml of ditercalinium at $OD_{600\ nm}$ = 0.1 in the same medium supplemented only with 2.5 μg/ml of thymine. At times indicated, a 50 μl aliquot was removed and 10 μl of [^{3}H]thymine or [^{14}C]uracil or [^{14}C]leucine was added (2 μCi/sample) to the aliquot. After 10 min of incubation at 33°C, the cells in the 50 μl aliquot were collected on 3 MM filters. Filters were treated and washed as described previously (23), except that for [^{14}C]uracil and [^{14}C]leucine incorporation the NaOH preloading of the filters was omitted. Radioactivity was counted with a Beckman scintillation counter (LS 1800).

At times indicated, aliquots of bacterial suspension treated or not with ditercalinium were diluted and poured on LBT plates for cytotoxicity determination.

Alkaline sucrose gradient sedimentation.

BL300 (lig7, thy^{-}) and BL301 (polA1, thy^{-}), strains which are mutants able to grow on low concentration of thymine, were used. Bacteria were grown

overnight at 33°C in M9 medium containing 10 µg/ml of thymine. Saturated cultures were diluted at 2 % in M9 medium containing 2.5 µg/ml of thymine plus either [^{3}H]thymine (60 µCi/ml) on [^{14}C]thymine (12 µCi/ml).

Bacteria were grown up to an $OD_{600\ nm}$ = 0.4.

Labeled bacteria were diluted at an $OD_{600\ nm}$ = 0.1 in M9 medium containing 50 µg/ml of non-labeled thymine. Samples were treated or not with ditercalinium (0.05 µg/ml) for 90 min either at 33°C or 42°C for BL300 strain or 120 min at 33°C for BL301 strain. After incubation spheroplasts were prepared and alkaline sucrose gradient sedimentation was performed as described (23).

RESULTS.

polA and lig7 mutations specifically confer sensitivity to ditercalinium.

The DNA polymerase activity of the BL102 strain originally isolated for ditercalinium sensitivity (9) was very low (< 5 % of the wild-type). This strain was also UV and MMS sensitive. These results suggested that the ditercalinium sensitivity of the BL102 strain was related to a polA mutation. This was confirmed by studying the behavior of authentic polA strains. Figure 1 shows the relative sensitivity to ditercalinium of polA1, polA12 at 42°C

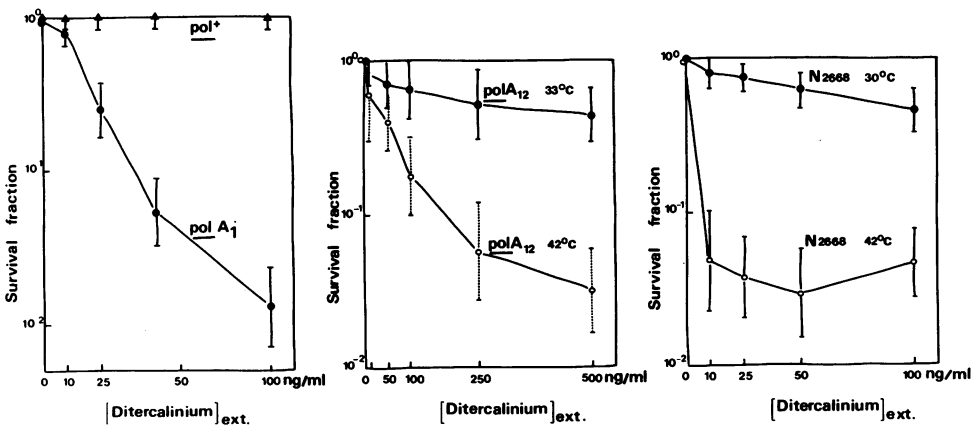

Figure 1. Survival of GC4716 (polA1), RH2456 (polA12) and N2668 (lig7) strains after ditercalinium treatment.
Bacteria were grown and treated as described in the Method section. RH2456 and N2668 strains were treated with ditercalinium 120 min at 42°C and plated at 33°C.

and 33°C and pol$^+$ strains. Furthermore, it was observed that all polA$^+$ revertants isolated from polA by selecting for the partial MMS resistance were all resistant to ditercalinium.

In addition, the lig7 strain which is deficient in ligase activity at high temperature is also ditercalinium sensitive (figure 1) whereas cells mutated in one of the following genes (uvrA, uvrB, uvrC, uvrD, recA, lexA, ruv) all involved in DNA repair, were completely resistant to ditercalinium.

Ditercalinium sensitivity of polA strains is reverted by uvrA mutation.

When polA cells which display ditercalinium sensitivity were plated on medium containing a high concentration of ditercalinium (10 µg/ml), ditercalinium resistant cells appeared at high frequency ($\simeq 10^{-4}$). More than 500 revertants colonies were tested for MMS sensitivity. They all retained the MMS sensitivity characteristics of the polA phenotype.

In addition, all these revertants were much more UV sensitive than the parental polA strain.

These results suggested that the ditercalinium resistance was obtained through the occurrence of an additional mutation in one of the genes involved in UV repair. The double mutants polA uvrA, polA lexA, polA12 uvrA, polA12 uvrC, polA12 uvrD were constructed and tested for their ditercalinium sensitivity. polA uvrB was known to be not viable (24) and therefore could not be studied. The double mutants polA12 uvrA and polA12 uvrD were found not viable at 42°C and the ditercalinium sensitivity could not be measured in non permissive conditions.

Figure 2 shows the relative sensitivity of the polA uvrA double mutant to ditercalinium as compared to polA strain. The polA uvrA double mutant appears completely resistant to ditercalinium. Contrastingly the polA12 uvrC double mutant has the same sensitivity as the polA12 mutant.

In addition, the presence of the uvrA mutation in polA strains which have become ditercalinium resistant after selection in presence of ditercalinium was tested by complementation. These ditercalinium resistant strains were lysogenized with the phage λ uvrA$^+$. Under these conditions, the lysogenized mutant strains are able to code for a functional uvrA protein. All the lysogenized polA ditercalinium resistant strains (7/7 tested) became ditercalinium sensitive. This further demonstrates that the polA sensitivity to ditercalinium can be suppressed by a uvrA mutation and can be restored by a functional expression of the uvrA protein.

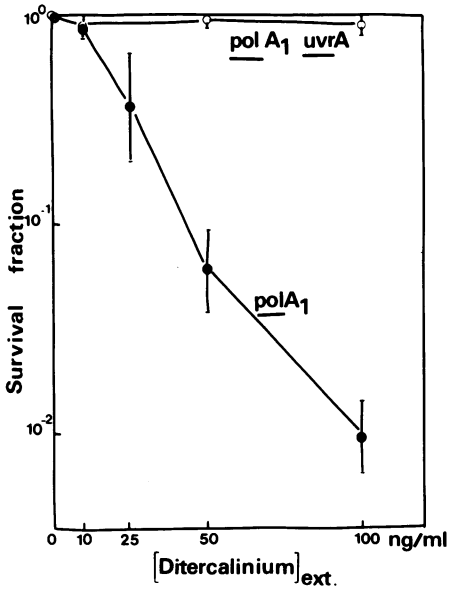

Figure 2. Survival of GC4716 (polA1) and BL501 (polA1 uvrA) strains after ditercalinium treatment.
Bacteria were treated as described in the Method section.

Ditercalinium induces DNA single strand breaks when ligase is inactivated at high temperature in lig7 mutant.

The lig7 strain is clearly more sensitive to ditercalinium at high temperature (42°C) than at low temperature (33°C) (figure 1). However at low temperature, ditercalinium elicits a small but significative toxicity on lig7 cells. This is probably related to the fact that even at low temperature, ligase activity is quite low in lig7 cells (24). DNA of lig7 cells which were treated with ditercalinium at 33°C and 42°C were analysed on alkaline sucrose gradient. Data shown in figure 3 demonstrate that in presence of ditercalinium, single strand breaks accumulated when ligase was inactivated at high temperature whereas breaks were not observed at low temperature. Surprisingly no breaks could be detected in polA1 strain treated with ditercalinium.

Ditercalinium induces the SOS function in polA1 strain.

It was observed that filamentation of the polA1 cells was induced by ditercalinium treatment. This suggested that ditercalinium could induce the SOS functions in polA1 cells. This was confirmed when induction of SOS func-

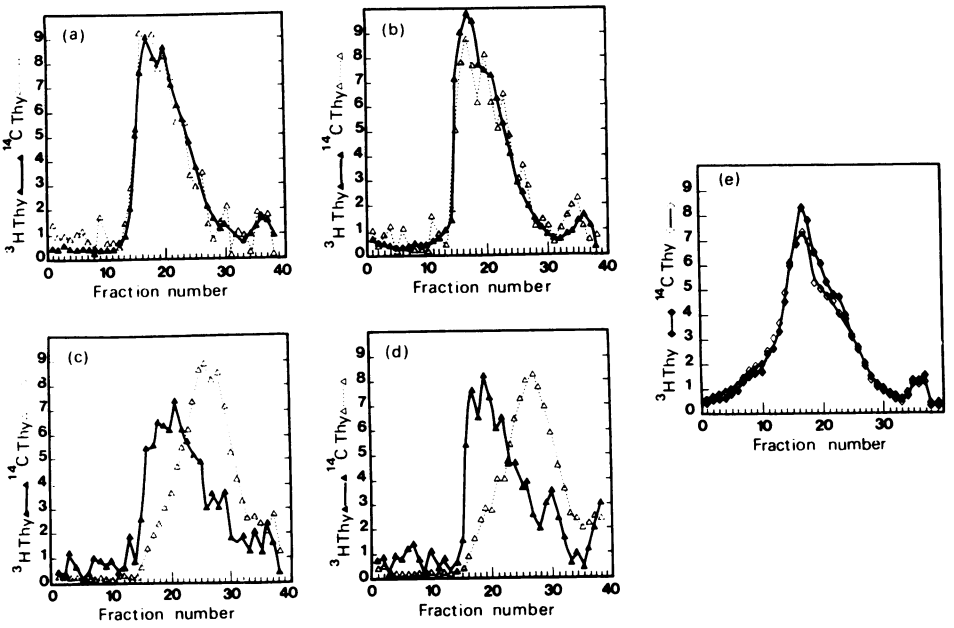

Figure 3. DNA analysis of ditercalinium treated cells by alkaline sucrose gradient sedimentation.
BL300 (lig7) was treated as in the Method section. 25 µl of spheroplasts from each treated or not treated samples were placed on the top of an alkaline sucrose gradient. Sedimentation was performed as described (23).
Results are expressed as the percentage of the total acid precipitable radioactivity.
Panel a : ▲ ▬▬ ▲ lig7 cells at 33°C, △ ····· △ ditercalinium treated lig7 cells at 33°C ; panel b : ▲ ▬▬ ▲ lig7 cells at 33°C, △ ····· △ lig7 cells at 42°C ; panel c : ▲ ▬▬ ▲ lig7 cells at 42°C, △ ····· △ ditercalinium treated lig7 cells at 42°C ; panel d : ▲ ▬▬ ▲ ditercalinium treated lig7 cells at 33°C, △ ····· △ ditercalinium treated lig7 cells at 42°C ; panel e : ◇ ▬▬ ◇ polA cells, ◆ ▬▬ ◆ ditercalinium treated polA cells.

tions was measured using the procedure of Quillardet et al. (22). In this method, the induction of β galactosidase synthesis is measured in lac⁻ E. coli strains lysogenic for λ(sfiA::lacZ). In this strain, expression of β galactosidase is under the control of an SOS dependent promoter. The polA1 strain lysogenized with λ(sfiA::lacZ) was used to measure the effect of ditercalinium on β galactosidase synthesis induction. The results of these measurements are shown in figure 4. Ditercalinium was found to induce β galactosidase synthesis in the polA1 strain, and therefore SOS functions at doses which are close to those which cause the death of polA cells. The induction of the SOS functions in that case cannot be related to an inhibi-

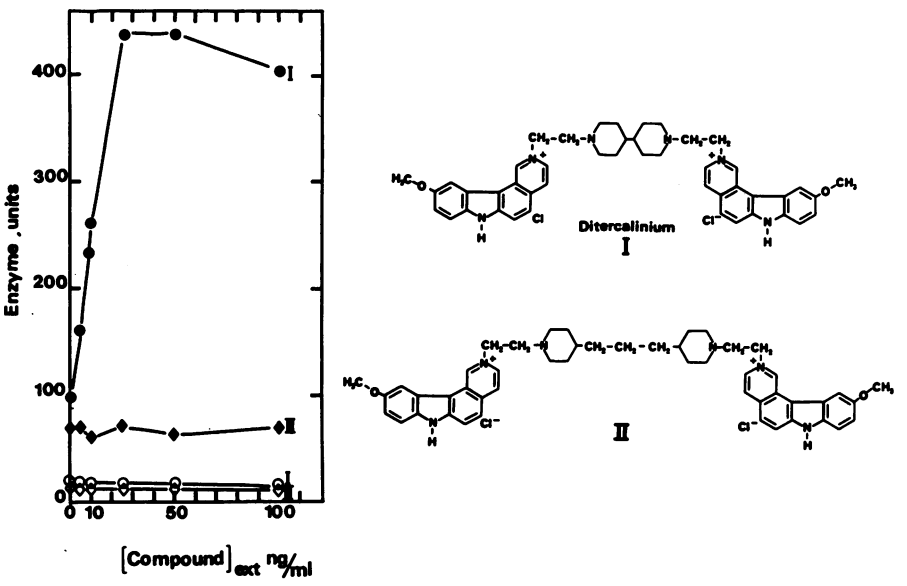

Figure 4. SOS inducing ability of ditercalinium and its analogue on GC4727 [polA1 λ(sfiA::lacZ)] and GC4718 [pol$^+$ λ(sfiA::lacZ)] strains.
●—●, ◆—◆ GC4727
○—○, ◇—◇ GC4718.
Bacteria were treated as described in the Method section.

tion of DNA synthesis. As shown in figure 5 no inhibition of DNA, RNA and protein synthesis is observed, even after 4 hours incubation with ditercalinium at concentrations able to induce SOS functions at the maximum rate.

Interestingly, a closely related ditercalinium analogue devoid of anti-tumor activity and cytotoxicity on mammalian cells, was unable to induce β galactosidase in this strain (figure 4) and did not elicit cytotoxicity on polA strains.

Because DNA breaks could not be detected in polA1 strain after ditercalinium treatment, it could be hypothetized that cell death resulted from uncontrolled induction of SOS functions by analogy with the situation encountered in thymineless induced death (25). To test this hypothesis, the effect of protein synthesis inhibition on ditercalinium toxicity in polA1 cells was measured. The results of figure 6 show that, indeed, protein synthesis inhibition by chloramphenicol significantly increases survival. To test whether some SOS functions are involved in lethality, ditercalinium

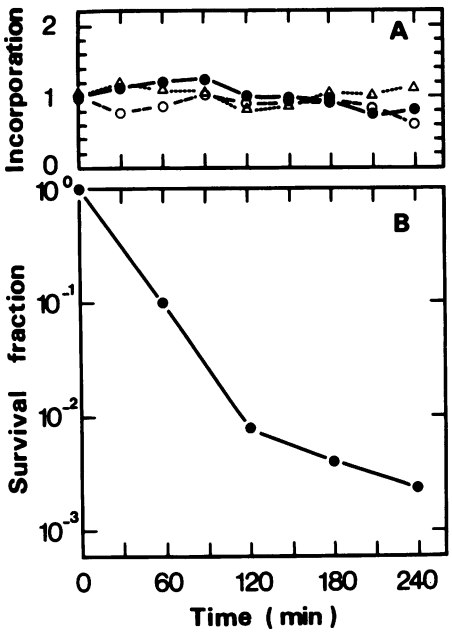

Figure 5. Ditercalinium effect on macromolecular synthesis and survival on BL203 (polA1 sfiA) strain.
BL203 strain was treated as described in the Method section.
Panel A : labeled precursor incorporation kinetics.
●—● [^{3}H]thymine
○—○ [^{14}C]uracil
△—△ [^{14}C]leucine
The results were standardized in comparison with labeled precursor incorporation in non treated cells.
Panel B : survival fraction of ditercalinium treated cells.

sensitivity of double mutant strains associating a polA mutation with a mutation in one of the genes involved in the SOS response was studied. The double mutant strains (polA1 sfiA, polA1 umuC, polA1 lon) and the strain polA1 sfiA sfiC were found to be as ditercalinium sensitive as the polA1 parental strain (results not shown). Contrastingly, polA1 lexA was found significantly more sensitive to ditercalinium (figure 7).

DISCUSSION.

Many bulky electrophilic molecules, such as alkylating agents and activated carcinogens, form covalent adducts with DNA. It is well established that these adducts can be removed inside E.coli and the native DNA structure

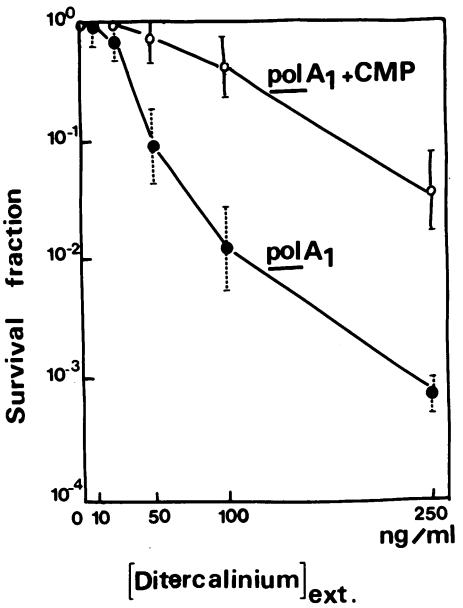

Figure 6. Effect of chloramphenicol treatment on the survival of ditercalinium treated GC4716 (polA1) strain.
Bacteria were grown and diluted to $OD_{600\ nm}$ = 0.1 (see cytotoxicity section). Bacterial suspension was separated in two samples. One sample was treated with 100 µg/ml of chloramphenicol, the other sample was incubated in absence of chloramphenicol. After 60 min of incubation at 33°C, bacterial suspension (2 ml in plastic bechers) was treated with various concentrations of ditercalinium. After 120 min of incubation at 33° C, bacteria were diluted 10 fold in $MgSO_4$ 10^{-2} M containing sonicated calf thymus DNA at a concentration 100 fold that of the ditercalinium. The presence of calf thymus was to complex free drug. After 60 min at 0°C, bacteria were diluted and poured on LBT plates for the determination of surviving fraction.

●—● no chloramphenicol
○—○ chloramphenicol treated cells.

restored through the concerted action of the proteins coded by the uvrA, uvrB, uvrC, uvrD, polA and lig genes (for review see Friedberg, 1985). In contrast, there is no report of non covalent DNA binding drug triggering a DNA repair response. This is well illustrated by the differential effect on DNA repair in E. coli of ethidium and ethidium azide in the dark which both bind reversibly to DNA and ethidium azide after its photoactivated covalent binding to DNA (27).

The results presented here show that ditercalinium, a bifunctional intercalator which forms a reversible and high affinity complex with DNA,

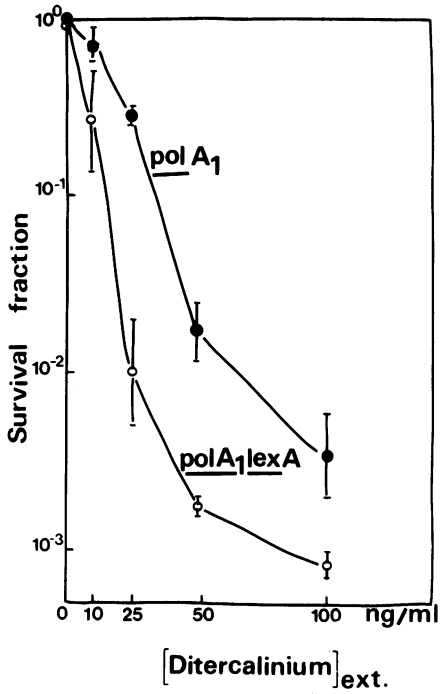

Figure 7. Survival of GC4716 (polA1) and BL507 (polA1 lexA) strains after ditercalinium treatment.
see legend of the figure 1.
● polA1
○ polA1 lexA.

interferes with the UV repair system. However, the interference with the UV DNA repair system is completely different from that observed in the case of covalent bulky adducts as shown by the following observations :

• Ditercalinium sensitivity was found specifically associated with the polA or lig mutations and not with any other single mutation tested (uvrA, uvrB, uvrC, uvrD, lexA, recA, ruv, lon, umuC, sfiA, sfiC) which are all involved directly or indirectly in DNA repair.

• The uvrA mutation which increases the sensitivity of polA cells to UV and compounds forming DNA covalent adducts (28) completely suppresses the toxicity of ditercalinium. The addition of a functional gene uvrA in ditercalinium resistant polA1 uvrA strain restores the ditercalinium sensitivity.

• The double mutant polA12 uvrC remains as ditercalinium sensitive as polA12 at high temperature. The double mutants polA12 uvrA, polA12 uvrD were found not viable at high temperature and could not be studied. polA1 uvrA does not grow at 41°C and this is therefore consistent with the fact that polA12 uvrA cannot grow at 41°C. In agreement with these observations, no reversion of ditercalinium resistance in any of the revertant strains isolated from polA1 strain was observed by complementation with the λ uvrC$^+$ phage.

• Although DNA single strand breaks are clearly observed in lig7 at 42°C in ditercalinium treated cells, no DNA lesions could be detected after ditercalinium treatment of the polA1 strain. No DNA repair synthesis was detected in $dnaA_{ts}$ at 42°C in ditercalinium treated cells but was detected in UV irradiated cells (data not shown).

• Neither DNA replication nor RNA and protein synthesis inhibition was observed even after 3 hours incubation with ditercalinium in the polA1 strain, an observation which is consistent with the absence of a DNA synthesis blocking lesion in this strain.

• Although DNA synthesis is not inhibited, SOS induction is clearly observed in the polA1 strain.

All these observations can be rationalized according to a model presented in figure 8.

Ditercalinium binds to DNA and induces a DNA conformational change recognized as a lesion by the uvrAB complex. UvrC binds later and DNA is incised (as single strand breaks are observed in lig7 at 42°C).

Ditercalinium analogues with long linking chains which could bind to DNA without inducing such conformational change could not be recognized and do not elicit any cytotoxic or SOS inducing effects.

In wild-type strains, polA and uvrD proteins could not excise the DNA fragment because of the large increase of DNA stability caused by the ditercalinium binding (7). This would account for the lack of unscheduled DNA repair synthesis in wild-type strain and the lack of gaps in DNA after ditercalinium treatment in the polA1 strain and the absence of immediate inhibition of DNA synthesis.

Because ditercalinium is not covalently bound, it will dissociate from the DNA-repairosome complex, leaving the repairosome bound to a DNA of normal structure. In wild-type environment polA and uvrD gene products increase the turnover of the DNA uvrABC complex (29,30). In presence of polymerase I,

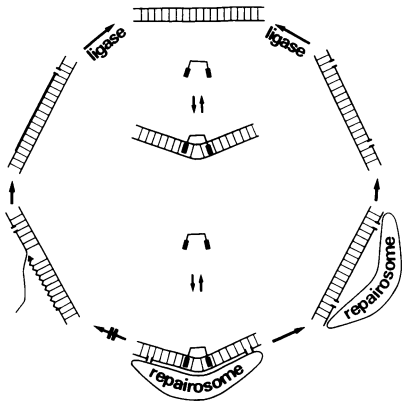

Figure 8. Proposed model for the action of ditercalinium. (see discussion in the text).

after ditercalinium is released, the repairosome bound to DNA of normal structure could dissociate and single strand breaks could be rapidly sealed by ligase, accounting for the observed presence of DNA breaks in lig7 mutant and its absence in the pol$^+$ strain. In the same conditions, but in absence of polymerase I, the uvrABC complex would remain bound to DNA for a much longer time. We propose therefore, that the presence of a uvrABC complex bound to a normal DNA structure is the factor responsible for cytotoxicity.

How such a factor is responsible for cytotoxicity is presently unknown. One could have thought that the uncontrolled SOS induction could lead to cell death. This could be suggested by the fact that ditercalinium cytotoxicity is decreased after chloramphenicol treatment.

However, the strains polA sfiA sfiC polA lon, polA umuC which associate polA- with a mutation involved in SOS response appears as sensitive to ditercalinium as polA. Furthermore, in the double mutant polA lexA, SOS functions cannot be induced. However, this strain appears significantly more ditercalinium sensitive than polA suggesting that some SOS function could contribute to the protection of the cells as observed for covalent adducts.

According to such a model, ditercalinium, by inducing a DNA conformational change similar to the one caused by covalent adducts, would act for the repair system as a dummy lesion because of its non covalent nature. Attempt to repair such a dummy lesion would lead to a futile DNA repair process lethal in a polA mutant. As expected in such a model, stopping this futile repair cycle by uvrA mutation in a polA strain completely suppresses ditercalinium toxicity.

These experiments reveal a very important property of DNA polymerase I. Its presence appears absolutely required to prevent toxic effects resulting from the non covalent binding to DNA of molecules which induce DNA structural modifications recognized by the repair incision complex. This property might be related to both its polymerase activity and its ability to increase the turnover of the repairosome accounting for the lack of uvrD effect. Most significantly, the toxicity of ditercalinium analogues in polA strains correlates with the cytotoxicity and the antitumor activity of these derivatives (unpublished results). Studies to analyse whether ditercalinium and its derivatives induce such processes in eucaryotic systems are presently in progress in our laboratory.

ACKNOWLEDGMENTS.

The authors are very grateful to Drs. R. D'Ari, R. Fuchs, M. Radman, R. Devoret, J. Laval, S. Boiteux, R. Sharma for helpful discussions. We wish to thank Drs. B. Bachman, P. Moreau, P. Quillardet for providing strains. D. Fortin is acknowledged for her technical assistance. We are grateful to Dr T. O'Connors to his help in editing the manuscript. This work was financially supported by CNRS, INSERM, Université Pierre et Marie Curie, l'Association pour le Developpement de la Recherche sur le Cancer and la Fondation de la Recherche Médicale.

REFERENCES.

1. Le Pecq, J-B., and Roques, B.P. (1986) in : Mechanisms of DNA damage and repair. Simic, M.G., Grossman, L., and Upton, A.C. (Eds) Plenum, New-York. Vol. 38, pp: 219-229.
2. Wakelin, L.P.G. (1986) Med. Res. Rev. 6, 275-340.
3. Laugâa, P., Markovits, J., Delbarre, A., Le Pecq, J-B., and Roques, B.P. (1985) Biochemistry 24, 5567-5575.
4. Pelaprat, D., Oberlin, R., Le Guen, I., Le Pecq, J-B., and Roques, B.P. (1980) J. Med. Chem. 23, 1330-1335.
5. Pelaprat, D., Delbarre, A., Le Guen, I., and Roques, B.P. (1980) J. Med. Chem. 23, 1336-1343.
6. Delbarre, A., Delepierre, M., Garbay, C., Igolen, J., Le Pecq, J-B., and Roques, B.P. (1987) Proc. Natl. Acad. Sci. USA, 84, 2155-2159.
7. Garbay-Jaureguiberry, C., Laugâa, P., Delepierre, M., Laalami, S., Muzard, G., Le Pecq, J-B., and Roques, B.P. (1987) Anticancer Drug Design, 1, 323-335.
8. Esnault, C., Roques, B.P., Jacquemin-Sablon, A., and Le Pecq, J.B. (1984) Cancer Res. 44, 4355-4360.
9. Lambert, B., and Le Pecq, J-B. (1982) C.R. Hebd. Seances Acad. Sci., Ser. C, 294, 447-450.
10. Elledge, S.J., and Walker, G.C. (1983) J. Mol. Biol. 164, 175-192.
11. Attfield, P.V., Benson, F.E., and Lloyd, R.G. (1985) J. Bacteriol. 164, 276-281.
12. Bichara, M., and Fuchs, R. (1987) J. Bacteriol., 169, 423-426.

13. Blingsmo, O.R., Steinum, A.L., Rivedal, E., and Seeberg, E. (1981) in Chromosome damage and repair, Seeberg, E., and Kleppe, K. (Eds.) Plenum, New-York, Vol. 40, pp: 303-309.
14. Howard-Flanders, P., and Boyce, R.P. (1966) Radiat. Res. 6 (suppl.), 156-184.
15. Trisler, P., and Gottesman, S. (1984) J. Bacteriol. 160, 184-191.
16. Gottesman, M.M., Hicks, M.L., and Gellert, M. (1973) J. Mol. Biol. 77, 531-547.
17. Monk, M., and Kinross, J. (1972) J. Bacteriol. 109, 971-978.
18. Blanco, M., and Pomes, L. (1977) Molec. Gen. Genet. 154, 287-292.
19. Huisman, O., and d'Ari, R. (1981) Nature 290, 797-799.
20. Miller, J.H. (1972) Experiments in Molecular Genetics (Cold Spring Harbor Laboratory, NY.).
21. Bochner, B., Huang, H.C., Schieven, G., and Ames, B.N. (1980) J. Bacteriol. 143, 926-933.
22. Quillardet, P., Huisman, O., D'Ari, R., and Hofnung, M. (1982) Proc. Natl. Acad. Sci. USA 79, 5071-5075.
23. Rupp, W.D., and Flanders, P.H. (1968) J. Mol. Biol. 31, 291-304.
24. Kornberg, A. (1980) DNA Replication, W.H. Freeman and Co, San Francisco.
25. Friefelder, D., and Levine, E. (1972) Biochem. Biophys. Res. Com. 46, 1782-1787.
26. Friedberg, E.C. (1985) DNA repair, W.H. Freeman and Co., New-York.
27. Lambert, B., Laugâa, P., Roques, B.P., and Le Pecq, J-B. (1986) Mutat. Res. 166, 243-254.
28. Monk, M., Peacey, M., and Gross, J.D. (1971) J. Mol. Biol. 58, 623-630.
29. Caron, P.R., Kushner, S.R., and Grossman, L. (1985) Proc. Natl. Acad. Sci., USA 82, 4925-4929.
30. Husain, I., Van Houten, B., Thomas, D.C., Abdel-Monem, M., and Sancer, A. (1985) Proc. Natl. Acad. Sci. USA 82, 6774-6778.